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Starter feed for carnivorous species as a practical replacement of bloodworms for a vertebrate model organism in ageing, the turquoise killifish *Nothobranchius furzeri*

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Abstract

The absence of a controlled diet is unfortunate in a promising model organism for ageing, the turquoise killifish (*Nothobranchius furzeri* Jubb, 1971). Currently captive *N. furzeri* are fed bloodworms but it is not known whether this is an optimal diet. Replacing bloodworms with a practical dry feed would reduce diet variability. In the present study, we estimated the nutritional value of the diet ingested by wild fish and determined the fish-body amino acid profile as a proxy for their nutritional requirements. We compared the performance of fish fed four commercial feeds containing 46%–64% protein to that achieved with bloodworms and that of wild fish. Wild fish target a high-protein (60%) diet and this is supported by their superior performance on high-protein diets in captivity. In contrast, feeds for omnivores led to slower growth, lower fecundity and unnatural liver size. In comparison to wild fish, a bloodworm diet led to lower body condition, overfeeding and male liver enlargement. Out of the four dry feeds tested, the fish fed Aller matched wild fish in body condition and liver size, and was comparable to bloodworms in terms of growth and fecundity. A starter feed for carnivorous species appears to be a practical replacement for bloodworms for *N. furzeri*. The use of dry feeds improved performance in comparison to bloodworms and thus may contribute to reducing response variability and improving research reproducibility in *N. furzeri* research.

KEYWORDS
African killifish, laboratory diet, nutritional ecology, practical diet

1 | INTRODUCTION

Diet standardization is fundamental for reducing organismal response variability and improving research reproducibility among different laboratories (Barnard *et al*., 2009; Reeves *et al*., 1993). However, the development of standardized or reference diets is one of the greatest challenges in the husbandry of laboratory organisms (Reeves *et al*., 1993). This is partly due to underestimation of the dietary standard by the research community and lack of knowledge of the nutritional requirements of the organism (Lawrence *et al*., 2012; Reeves *et al*., 1993). This lack of standardized diet is problematic in experimental fish.

Life-stage specific nutritional requirements for recently introduced model organisms are poorly known and finding the basal life-sustaining and growth-supporting nutritional requirements are primary goals during the early stage of standardized or optimal feed development (Jobling, 2016; Watts *et al*., 2016). The macronutrient composition of feeds as well as their fatty and amino acid composition have a profound effect on the reproductive and somatic performance of fish (Dabrowski & Guderley, 2003; Meinelt *et al*., 1999). In addition,

it is suggested that whole-body amino acid composition may reflect fish amino acid requirements (Akiyama *et al*., 1997; Snellgrove *et al*., 2011; van der Meer & Verdegem, 1996). Thus, knowledge of the fatty and amino acid concentrations in both feeds and the fish body is valuable for optimizing fish performance.

Increase in mass and body size are not satisfactory measures for estimating fish health and more nuanced examination is necessary (Watts *et al*., 2016). Histology is a useful tool for assessing the health status of fish due to known associations of specific feeds and degenerative changes of the digestive tract (Hedrera *et al*., 2013; Heikkinen *et al*., 2006), liver vacuolization (Žák *et al*., 2020) and tumorigenesis (Spitsbergen *et al*., 2012; Watts *et al*., 2016). Also diet significantly influences the size of organs involved in food processing, such as the gut (Zandona *et al*., 2015) and liver (Ruohonen *et al*., 2007). Knowledge of how the gross morphology of organs is affected by diet is especially important in laboratory organisms; laboratory animals' organ size should be as natural as possible (Spitsbergen *et al*., 2012; Watts *et al*., 2016). Thus a crucial step in identifying the best laboratory feed is the comparison of diet-dependent laboratory outcomes to the performance of wild fish to identify unnatural performance caused by suboptimal feed and delineate the 'healthy phenotype' (Watts *et al*., 2016).

The turquoise killifish *Nothobranchius furzeri* (Jubb 1971) is a recently introduced model organism for biomedicine, evolutionary biology and toxicology, and has been kept in the laboratory for less than two decades (Genade *et al*., 2005; Hu & Brunet, 2018; Philippe *et al*., 2018; Reichard & Polačik, 2019). Despite its increasing importance, there is no consensus on what should be used as standard feed (Reichard *et al*., 2022). The role of standard *N. furzeri* nutrition in research appears to have been neglected despite its significant role in research outcomes (Terzibasi Tozzini *et al*., 2009; Vrtílek & Reichard, 2015). *N. furzeri* were initially considered to be reluctant to consume dry feed (Polačik *et al*., 2016) but recently it has been found that they both accept and thrive on it (Harel & Brunet, 2016; McKay *et al*., 2021; Žák *et al*., 2020). Still the most frequently used feed for *N. furzeri* husbandry is bloodworms, chironomid larvae which are often collected in the wild (Dodzian *et al*., 2018; Muck *et al*., 2018; Polačik *et al*., 2016).

The ultimate goal with laboratory fish should be to limit live food as much as possible in order to reduce variability in the response to treatments, standardize outcomes among laboratories, control nutritional quality, prevent seasonal availability and avoid risk of disease introduction (Armitage, 1995; Barnard *et al*., 2009; Lawrence *et al*., 2012; Watts *et al*., 2012). In addition, the laboratory diet should be easily deliverable and its preparation not labour intensive. A bloodworm diet fails most of these criteria. Even minor changes in feed formulation can affect gene expression (Williams & Watts, 2019), hence the need for diet standardization in a genetically tractable model organism such as *N. furzeri* (Hu & Brunet, 2018).

The aims of this study were (a) to estimate *N. furzeri* nutritional requirements by determining its amino acid and fatty acid body composition, (b) to compare the performance of *N. furzeri* fed various commercially available feeds varying in proportion and source (animal based *vs*. plant based) of protein, (c) to compare performance on these diets with that achieved on bloodworms and (d) to compare fish performance on these diets to that of their counterparts in the wild. The study will provide important insights into the nutrition of captive *N. furzeri* and indicate further steps towards developing an appropriate laboratory diet.

2 | MATERIALS AND METHODS

2.1 | Ethical statement

Experimental procedures, handling protocols and facility were approved according to the laws of the Czech Republic No. 246/1992 and No. 419/2012, and by Ministry of Agriculture (breeding facility No. CZ 62760203, permit approval document 62,116/2017-MZE-17214). All fish were killed by overdose of anaesthetics at the end of the experiment for tissue sampling.

2.2 | Fish husbandry

Fish from a wild derived population MZCS 222 of *N. furzeri* (Cellerino *et al*., 2016) were hatched on 26 October 2020. At 18 days post hatching (dph) they were transferred from a communal aquarium into 10 9 l aquaria in a recirculating system (RS). Initial density (18–21 dph) was 20 individuals per 9 l aquarium, reduced to 10 individuals per 9 l at 21 dph and eight individuals per 9 l at 53 dph as fish grew. The water temperature was 26.8 ± 0.36°C (mean ± S.D.), monitored by two temperature loggers (HOBO UA-002–64, Onset Computer, Bourne, MA, USA) with a 4 h logging interval. The bottom of the aquarium was siphoned daily and 25% of RS water was replaced every 10 days. The light regime was 14 h light:10 h dark (light 6:00–20:00). Each aquarium contained both sexes and each diet was used in four replicates (aquaria). The experiment was terminated after 91 dph. Water parameters and further husbandry details may be found in Supporting Information S1.

2.3 | Experimental feeding

Juveniles were fed three times per day with freshly hatched *Artemia* nauplii (24 h from salt-water incubation; Sanders, Ogden, USA, www.gsla.us) from hatching until 18 dph. From 18 to 21 dph (average body size 18 ± 1.4 mm standard deviation (SD)) *Artemia* were supplemented with either dry feed (0.4 mm pellet size, for dry feed groups) or ground bloodworms (defrosted Chironomidae larvae in the bloodworm group). After 21 dph all fish were fully weaned onto their experimental diet.

Five experimental diets were compared. Two dry feeds for carnivorous fish species, Aller Infa 0.4 mm (ALR, starter feed; Aller Aqua, Germany) and Skretting Vittalis 2.5 mm (SKR, grow-out feed; Skretting, Norway), and two diets for omnivorous species, SAK MIX, 0.4–0.6 mm (SAK, pet-fish feed; Exot Hobby, Czech Republic) and Coppens Orange 3 mm (COP, broodstock feed; Alltech, Netherlands),

were used as experimental dry feeds (see Supporting Information S2 for feeds ingredients list). Defrosted bloodworms (BLW; Grýgera, Czech Republic, www.nakrmryby.cz) served as a control as this is the diet currently used across laboratories using *N. furzeri* as a research model. The selected feeds cover a wide range of crude protein concentration (46%–64%) and are relatively low on lipids (<11%), which is recommended for warm-water species (NRC, 1977; see Supporting Information S3 for proximate composition reported by manufacturer). Aquaculture feeds (ALR, COP, SKR) were used in preference to pet-fish feeds (SAK) because they are more refined, specifically tested for appropriate performance of target fish and monitored for contaminants because they are used for production of organisms for human consumption (Verstraete, 2013). Performance of experimental fish was compared to wild fish where published data were available.

All dry feeds were cold-repelletized to standardize their density (see Table 1 for density and Supporting Information S4 for the cold-repelletization procedure). From 18 to 24 dph fish in the dry feed groups were hand-fed with 0.4 mm pellets to satiation three times daily (11:30, 15:30, 19:30). From 25 dpf pellet size was increased to 0.6–1.4 mm and fish were fed twice daily (12:00 and 19:00). From 37 dph until the end of the experiment at 91 days fish were fed once per day around noon and after 74 dph 10% of pellet volume was replaced with larger pellets (1.6–2.2 mm).

2.4 | Macronutrient, fatty acid and amino acid composition

The proximate macronutrient composition of experimental diets was determined by the accredited laboratory at the State Veterinary Institute in Olomouc, Czech Republic (https://www.svuolomouc.cz/) and the results are reported in Table 1. The fatty acid analysis was done following established laboratory protocols (Mráz & Pickova, 2009). The amino acid analysis was done in an accredited third-party laboratory using ion chromatography and ISO certified protocols (Agrola s.r.o., Czech Republic). All calculations and results were based on dry matter. The proxy of optimal amino acid composition for *N. furzeri* was determined in accordance with the ideal protein concept (Rollin *et al*., 2003). Bloodworm-fed fish were chosen for amino acid analysis because they are considered the current laboratory standard (Dodzian *et al*., 2018; Philippe *et al*., 2018; Polačik *et al*., 2016) and fresh samples from the wild were impossible to acquire.

2.5 | Nutritional parameters

To compare the macronutrient composition of the diet of wild fish with that of the experimental feeds, crude protein and crude lipid content were estimated from published studies of the diet of wild *N. furzeri* (Polačik & Reichard, 2010; Žák *et al*., 2019). The macronutrient composition of dietary items occurring in the digestive tract of wild fish was estimated from published values (Supporting Information S5) and the relative contribution of the proteins and lipids of each dietary item was estimated from site-specific average representation of the respective dietary items.

A diet specific food conversion ratio (FCR) of experimental feeds was assessed at 10–14 day intervals from the amount of food consumed and body mass gain (both sexes pooled) in accordance with the formula FCR = food intake/body mass gain. The overall diet-specific and age-specific amount of food was measured during the whole course of the experiment from 21 to 91 dph and is presented in Supporting Information S6.

The sex-specific amount of food consumed (per body mass unit) was determined during female isolation preceding experimental spawning at 77–80 dph. The aquarium-specific amount of feed consumed/provided was determined by the difference between food mass in the stock container before and after feeding. In the case of

TABLE 1 Proximate macronutrient composition of the experimental diets used in the dietetic trial with *Nothobranchius furzeri*

	Bloodworm, BLW	Aller Infa, 0.4 mm ALR	Skretting Vitalis, 2.5 mm SKR	Coppens Orange, 3 mm COP	SAK mix, 0.7–1 mm SAK
Crude protein (%)	50.56 ± 3	66.95 ± 3	60.77 ± 3	48.26 ± 3	52.24 ± 3
Crude fat (%)	2.81 ± 8	9.18 ± 8	12.49 ± 8	6.37 ± 8	7.11 ± 8
Moisture (%)	85.80 ± 2	7.39 ± 2	4.73 ± 2	7.37 ± 2	5.71 ± 2
Carbohydrate (NFE, %)	21.77 ± 20	11.67 ± 20	13.36 ± 20	33.30 ± 20	25.92 ± 20
Ash (%)	21.77 ± 2.5	11.88 ± 2.5	12.81 ± 2.5	9.61 ± 2.5	11.98 ± 2.5
Fibre (%)	2.81 ± 10	0.31 ± 10	0.60 ± 10	2.43 ± 10	1.76 ± 10
Total phosphorus (%)	0.44 ± 5	1.65 ± 5	1.62 ± 5	1.35 ± 5	1.47 ± 5
Density (g ml^{-1}) original/repalletized	NA/NA	0.48[a] /0.58	0.59/0.60	0.47/0.60	0.55/0.64
Gross energy (kcal × 100 g^{-1})	314.6	397.1	408.9	383.6	376.6
Phosphorus:nitrogen ratio (mg × g^{-1})	54.4	154	166.6	174.8	175.9
Energy:protein balance[b]	2.2	1.9	2.7	3.9	3.2

Note. NFE, nitrogen-free extract, only a proxy of carbohydrate content. Value shown with diet name stands for original (nonrepelletized) pellet size. The pellet size of all the feeds was standardized for the feeding trial by repelletization (for details see Material and Methods).

[a]Density for nonrepelletized Aller Infa is estimated based on a pellet size of 0.4 mm, which may confound the density outcome due to different pellet sizes (0.5–1 mm) of other dry feeds.

[b]Energy: protein balance = nonprotein energy (NPE) to protein ratio (NPE: protein ratio (cal × mg^{-1})).

bloodworms, the frozen block was melted and sieved, and the wet mass measured before and after feeding. Fish were fed in several small doses to satiation and feeding ceased when they stopped reacting to food. There was no uneaten food after 5 min from the last small dose. The mass of food consumed was divided by the number of fish in each aquarium. There were two replicates (aquaria) for each 'sex–diet' combination (10 combinations) per day (4 days), giving a total sample of $N = 80$. The body mass of each female was assigned to its body size from the length–weight relationship (L–W, estimated at 80 dph). The sex-specific amount of food consumed by wild fish was taken from Žák *et al.* (2019) and was determined in the laboratory from formalin-fixed specimens.

The gut passage rate and solid waste production (proportion of faeces produced from a known amount of food) were determined at 86 dph by siphoning out the solid waste from the bottom of the aquarium at four time intervals (4, 8, 12 and 24 h post-feeding). All uneaten food was siphoned out 10 min after feeding. The waste produced was siphoned through a 5 mm pipe into a single 1 l bottle from all aquaria for each treatment and the bottle content was filtered through a 15 cm diameter cone-folded filtration paper (80 g m^{-2}). All filtration papers were oven dried (60°C for 12 h; Venticell 55, BMT, Czech Republic) and weighed (precision 0.001 g) prior to filtration. After waste siphoning, seven replicates of an equal amount of clean system water were filtered to determine the mass contribution of system water to the dried filtration paper. Filtration papers with waste were dried for 20 h at room temperature and then oven dried (60°C, 12 h). The solid waste weight was determined as the difference between the dry mass of filtration paper with faeces and clean filtration paper plus 0.0284 g (mean mass contribution of system water). It should be noted that the soluble metabolites from food digestion were neglected.

2.6 | Growth

Body size (SL, standard length in mm) and body mass (g) were measured at 10 day intervals from 19 to 49 dph and at 14 day intervals thereafter until the end of the experiment (91 dph). Body size was measured from photographs in shallow water with a millimetre scale at the bottom of the plastic container using ImageJ v1.48. Wet body mass was measured to the nearest 0.01 g by wiping each fish with a wet towel in a hand net before placing on the balance (PCB 350–3, Kern, Germany). Visual representations of body size growth for wild fish were used from Vrtílek *et al.* (2019). The thermal unit growth coefficient (TGC) was used as the least biased growth parameter (Lugert *et al.*, 2016), estimated from sex-specific differences in mean body size from two measurements. The formula was used in accordance with Cho (1992) and Lugert *et al.* (2016).

2.7 | Health indicators and other somatic parameters

Fish survival was recorded daily and dead fish were immediately removed. As a captive diet may contribute to the development of bone deformities (Lall & Lewis-McCrea, 2007), bone deformities presence was assessed at the end of the experiment. Bone deformities are rarely observed in the wild (~1:3000; Žák, pers. obs.) and thus their occurrence was estimated as zero for comparison with other diets.

Body condition is a frequently used health parameter, therefore residuals from the L–W relationships at the end of the experiment were used as a less biased condition parameter than the traditional Fulton's condition factor (Bentley & Schindler, 2013). L–W data (and consequently condition) for wild fish were taken from Vrtílek *et al.* (2018) and Žák *et al.* (2019), four sites, $N = 32$, all randomly selected. To avoid bias due to different gut fullness or preservation in wild fish samples, a complementary analysis with sex-specific L–W (eviscerated body mass) relationship was completed from the same individuals.

Several somatic parameters were determined at the end of the experiment (91 dph). The hepato-somatic index (HSI) was measured as the ratio of liver wet mass to eviscerated wet body mass. Wild HSI data were taken from Vrtílek *et al.* (2018) and the Institute of Vertebrate Biology (IVB) collection (unpublished data), four sites, 24 fish to obtain a balanced dataset. The visceral fat score was visually assessed based on the coverage of internal organs with adipose tissue (scale 0–3: 0, no visceral fat; 3, internal organs completely covered with fat). Gut length was also measured as this can be affected by diet (Zandona *et al.*, 2015). Gut length data for wild fish were taken from Žák *et al.* (2019), one site, four samplings, 24 fish.

2.8 | Histology

We performed a histological assay of the effect of diet on gut, liver and kidney from eight fish from each dietary treatment (one male and one female for each replicate). Fish were euthanized with an overdose of clove oil and their organs were immediately fixed with Lillie's F.A.A. (formalin–acetic acid–alcohol). The standard paraffin technique was applied. Histological sections were stained with Mayer's haematoxylin and eosin.

In the liver ($N = 40/39$ sampled/evaluated undamaged slides), the relationship of diet and hepatocellular vacuolation was scored in accordance with Di Cicco *et al.* (2011). The morphological type of vacuolation (glycogen-like, lipid and mixed) was determined in accordance with Wolf and Wolfe (2005). For comparison with wild fish, hepatocellular vacuolation data were taken from Vrtílek *et al.* (2018). Kidneys ($N = 40/31$) were checked for nephrocalcinosis as a marker of dietary homeostasis disruption (Ferguson, 2006). The gut ($N = 40/39$) was checked in transverse and longitudinal sections to record potential dystrophic or inflammatory lesions. To obtain comparative data from wild fish, the guts of 12 fish from four sites (IVB collection, unpublished) were investigated for the presence of lesions.

2.9 | Reproductive parameters

Reproductive allotment is significantly influenced by diet (Vrtílek & Reichard, 2015) and thus the gonado-somatic index (GSI) was determined as the relative wet mass (%) of ovaries in relation to

eviscerated wet body mass at age 91 days. GSI values of wild fish were taken from Vrtílek *et al.* (2018), four sites, 12 females. Fecundity (total number of eggs produced) and fertilization rate (proportion of fertilized eggs) were determined from pair spawnings of fish which were isolated for 4 days in sex-specific aquaria to standardize egg retention in female ovaries. Experimental spawning was conducted in 2 l containers with a 0.5 cm layer of fine sand and took 2 h, which is sufficient for oviposition of all ovulated eggs (Polačik *et al.*, 2016). Spawning aquaria were placed in the experimental recirculation system and each compartment had water inflow (0.25 l × min^{-1}). One week before the experimental spawning, fish were habituated to the spawning setup for 2 h. Eggs were sieved from the sand after 24 h and the total number of eggs and proportion of fertilized eggs were determined. Wild female fecundity was estimated as the number of ovulated eggs in ovaries and data were taken from Vrtílek *et al.* (2018), four sites, 12 females.

Subsets of fertilized eggs were taken during habituation spawning and experimental spawning (total $N = 599$) and their 31 dpf survival and developmental rate (diapause stage DII, slow developing, and further than DII, fast developing; Polačik *et al.*, 2014) was recorded. Eggs were incubated individually in a 96-well microtiter plate in Yamamoto solution (Blažek *et al.*, 2013) and checked under a stereomicroscope at 4–6 day intervals for 31 dpf.

2.10 | Statistical analysis

Overview, structure and sample size for all models may be found in Supporting Information S7. A Gaussian linear model (LM) was used for analysis of FCR, TGC and body condition (a mixed model was not used for body condition because use of the aquarium as a random factor led to a singular model). A linear model with permutation test (LMP, lmPerm v 2.1.0; Wheeler & Torchiano, 2016) was used for analysis of age dependent relative amount of food consumed. A Gaussian generalized additive model (GAM, mgcv v 1.8.33; Wood, 2017) was used for analysis of body size and body mass growth trajectory. An ordinal GAM (+1 was added to all values to avoid zeroes) was used for analysis of extent of hepatocellular vacuolation and visceral fat score. Morphological type of hepatocellular vacuolation was analysed with Pearson's chi-squared test.

A linear mixed effect model (LME, lme4 v 1.1.26; Bates *et al.*, 2015) was used for analysis of initial body mass (g, at 19 dph), proxy of juvenile growth (g, body mass at 29 dph), relative gut length (% of SL), hepato-somatic index (HSI %) and gonado-somatic index (GSI %). A negative binomial Generalized Linear Mixed Model (GLMM) was used for analysis of fecundity (all eggs produced by female). A binomial GLMM was used for analysis of fertilization rate (raw number of fertilized and unfertilized eggs) and developmental trajectory (number of post DII eggs, number of DII eggs at 31 dpf). Egg survival over 31 dpf was assessed using a Cox mixed model (coxme v 2.2.16; Therneau & Grambsch, 2000).

The proportion of bone deformities was analysed with Fisher's exact test, which handles low sample sizes. The wild and bloodworm treatment groups as well as SAK and Aller groups had identical proportions of deformities and thus were pooled. A Bonferonni corrected Fisher's exact test was used for *post hoc* comparisons (five comparisons, corrected $\alpha = 0.01$; McDonald, 2014).

All GAMs were checked for concurvity and were validated by visual evaluation of gam.check plots. All linear models were checked for multicollinearity by car::VIF (car v 3.0.10; Fox & Weisberg, 2019) and residuals were checked with diagnostic plots. All pairwise comparisons were performed by emmeans v 1.5.4 (Lenth, 2021). All statistical analyses were conducted in R environment v 4.0.4 (R Core Team, 2021).

3 | RESULTS

3.1 | Nutritional parameters

Wild fish target approximately 60%–63% of crude proteins and 10%-17% of crude lipids in the dry matter of their diet (Figure 1a). Eighteen amino acids analysed altogether comprise 97%–98% of *N. furzeri* body protein (Supporting Information S5). Among the essential amino acids (EAAs) which the body cannot produce, lysine seems to be required in the highest quantity (~7% of body protein). The ideal protein concept for *N. furzeri* was constructed around lysine and compared with the protein composition of the experimental diets (Supporting Information S8). Bloodworm (BLW) oversupplied the majority of amino acids despite being limited in sulphur-containing amino acids (methionine and cysteine). SAK had the worst amino acids profile (undersupplied amino acids = 7, oversupplied = 0; Supporting Information S8) exhibiting the worst performance in the trial (presented below). The aquaculture feeds (ALR, COP, SKR) had a suitable amino acid profile overall, providing surplus amino acids (average from 11.4% to 37.9%) in contrast to the SAK (negative deviance, average 9.9%). Skretting contained a somewhat lower amount of histidine (an EAA) which was probably manifested through poor fertilization rate and survivability of eggs despite reasonably good somatic growth (presented below).

In terms of fatty acid composition, all dry feeds (ALR, SAK, COP, SKR) contained a similar or surplus amount of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) than normally stored in the killifish body (Supporting Information S9). Even among PUFAs, the amount of omega fatty acids (>0.6% ω-3, >1% ω-6) exceed that in the killifish body (~0.62% ω-3%, 0.46% ω-6). BLW on the other hand provided only a restricted supply of PUFAs, ω-3 and ω-6 fatty acids compared to other dietary components (Supporting Information S9).

The FCR was poorest in the BLW group (LM, pairwise t-ratio = 10.74 to 12.30, $P < 0.001$; Figure 1b and Supporting Information S10). The differences among dry feeds were not significant (pairwise t-ratio = −1.57–1.52, $P = 0.534$–1.000; Figure 1b). Six times as much food was necessary to satiate the BLW group as the dry feed groups (Figure 1c).

Females and males differed in the amount of food consumed (LME, diet:sex interaction, $\chi^2_5 = 72.10$, $P < 0.001$; Figure 1c). Males

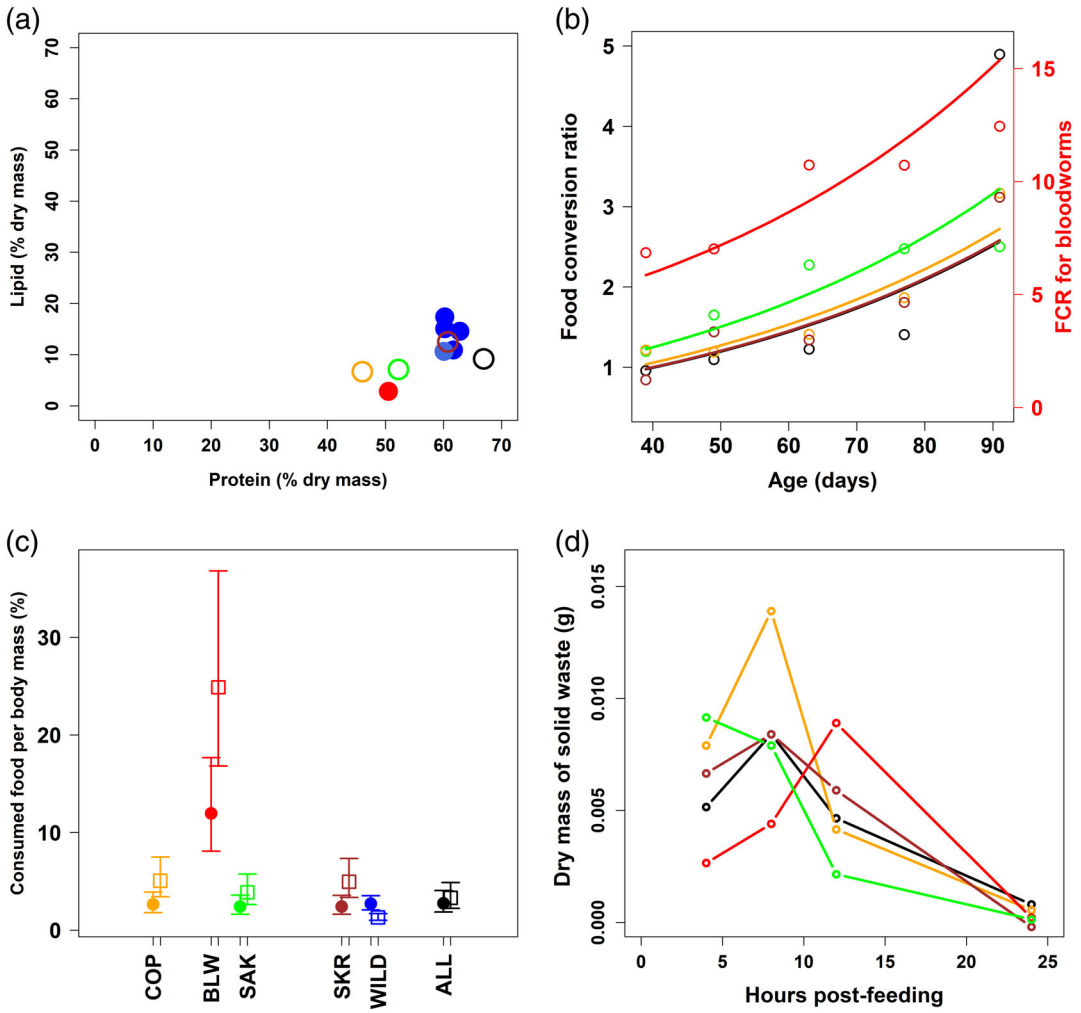

FIGURE 1 Nutritional parameters of *Nothobranchius furzeri* from the wild and the laboratory-fed pelleted food/bloodworms. (a) Targeted macronutrient composition of diet and macronutrient composition of experimental feeds in two-dimensional macronutrient space (protein:lipid). (○) Aller Infa; (●) bloodworms; (○) Coppens Orange; (○) SAK MIX; (○) Skretting Vitalis; (●) wild fish (Polačik & Reichard, 2010); (●) wild fish (Žák et al., 2019). (b) Age-dependent food conversion ratio of experimental diets. Note that the bloodworm FCR has a separate axis. Curves were produced from the linear model, back transformed log-FCR. Points represent observations. (—○—) Aller Infa; (—○—) bloodworms; (—○—) Coppens Orange; (—○—) SAK MIX; (—○—) Skretting Vitalis. (c) Sex-specific amount of consumed feed at 77–80 dph. Means and 95% confidence intervals (error bars) are linear mixed effect model estimates. The x axis is the crude protein concentration of diet in dry matter (Ruohonen et al., 2007). (□) male; (●) female. (d) Time-dependent amount of solid waste produced by fish siphoned from experimental aquaria over 24 h. (—○—) Aller Infa; (—○—) Skretting Vitalis; (—○—) Coppens Orange; (—○—) SAK MIX; (—○—) bloodworms. Points represent time of sampling and values are competed on solid waste mass produced by one fish per hour

consumed 92%–108% more food in the COP, SKR and BLW groups ($P = 0.013$–0.024; Supporting Information S11). SAK-fed (60%) and Aller-fed (20%) males consumed marginally more than females ($P > 0.088$). The opposite relationship was found in the wild fish, with females consuming 107% more food than males ($P < 0.001$; Supporting Information S11). There was no clear age-dependent trend in food consumption relative to body mass in adult fish (30–91 dph, LMP, $F_{1,24} = 2.98$, $P = 0.097$; Supporting Information S12).

Fish emptied their guts almost completely within 24 h, with a peak at 8 h post-feeding in pelleted groups and at 12 h post-feeding in the BLW group (Figure 1d). The lowest proportion of solid waste was produced by the ALR and BLW groups (9% and 8%, respectively) followed by SKR (13%), COP (16%) and SAK (17%).

3.2 | Growth

The growth trajectory (SL) was significantly influenced by diet in a sex-specific manner (Gaussian GAM, diet:sex interaction, $F_{4,1030} = 7.346$, $P < 0.001$; Figure 2a and Supporting Information S13 and S14). Aquaculture feeds followed a similar growth trajectory with the BLW group but not with SAK-fed fish, which grew slowly (Figure 2a). In

general, growth in body length is considerably slower in captivity than in the wild (Figure 2a). Age-dependent body mass and juvenile growth corresponded to the aforementioned trends (Supporting Information S15 and S16). Initial body mass (19 dph) was similar among groups (LME, $\chi^2_4 = 0.22$, $P = 0.995$).

Thermal growth coefficient (TGC) decreased with age (Gaussian LM, log-transformed estimate [Standard Error (SE)] −0.028 [0.003], $t = -9.55$, $P < 0.001$) and was lower in females (−0.694 [0.124], $t = 5.61$, $P < 0.001$). There was a tendency for TGC to be diet-dependent ($F_{4,53} = 2.44$, $P = 0.058$) with superior growth in the ALR group in comparison to SAK (pairwise ratio [se] 1.745 [0.341], $t_{53} = 2.847$, $P = 0.047$; Supporting Information S17 and Figure 2b). Other pairwise diet comparisons were not significant ($P > 0.127$; Supporting Information S17). Poor growth realized under SAK is apparently related to the amino acid composition of the diet, as mentioned above.

3.3 | Health indicators and other somatic parameters

Fish survival was similar in all dietary treatments (range 72%–82%, coxme, $\chi^2_4 = 2.27$, $P = 0.685$) for both males and females (males 75%, females 80%, $\chi^2_1 = 0.94$, $P = 0.332$; Supporting Information S18). The Aller and SAK groups had a significantly higher proportion of deformities (22% both) than the BLW or wild group' (Fisher's exact test, 0% both, $P < 0.001$). The COP and SKR groups were not significantly different from the BLW and wild groups (COP 16%, $P = 0.051$; SKR 6%, $P = 0.130$) or SAK and ALR (SKR, $P = 0.080$; COP, $P = 0.591$). Jaw deformity was the most frequently observed type of deformity (Supporting Information S19 and S20). The highest body condition was found in wild fish and it was similar to the ALR fish (pairwise estimate [se] 0.042 [0.25], $t_{176} = 1.72$, $P = 0.521$; Figure 2c). All other experimental groups had lower body condition ($P < 0.027$; Supporting Information S21). Very similar results were found when using eviscerated body mass instead of total body mass (Supporting Information S22).

Hepatocellular vacuolation was highest in the COP and BLW groups, in striking contrast to the low hepatocellular vacuolation of wild fish (ordinal GAM, pairwise estimate [se], COP-wild 6.34 [1.36], $t_{37.2} = 4.64$, $P = 0.001$; BLW-wild 4.88 [1.36], $t_{37.2} = 3.58$, $P = 0.012$; Figure 2e). Other dry feeds did not differ in vacuolation from wild fish ($P = 0.123$–0.615; Supporting Information S23). Overall, hepatocellular vacuolation was significantly higher in males than in females (5.32 [0.77], $z = 6.92$, $P < 0.001$; Figure 2e). The morphological type of vacuolation did not depend on diet (chi-squared test, $\chi^2_4 = 6.99$, $P = 0.136$). No unnatural tissue alterations were apparent during histological examination of gut and kidneys (Supporting Information S24).

Diet significantly affected the hepato-somatic index in a sex-specific manner (LME, diet:sex interaction, $\chi^2_5 = 46.72$, $P < 0.001$). Differences between male and female HSI were small in omnivorous (COP, SAK) diets and the BLW diet, in a striking contrast to wild fish and carnivorous (ALR, SKR) diets (Figure 2d and Supporting Information S25). The livers of BLW males were the largest among all groups and twice the size than observed in wild males ($P < 0.010$; Figure 2d, and Supporting Information S25). Visceral fat score was dependent on diet and was higher in males (Figure 2f and Supporting Information S26). Gut length was not affected by diet (LME, $\chi^2_5 = 7.58$, $P = 0.181$; Supporting Information S27) and females had longer guts than males, 3.9% of body size (se = 1.1%, $t = 3.7$, $P = 0.001$). Gut fullness significantly influenced gut length (estimate [se] 0.001 [0.0004], $t = 2.209$, $P = 0.027$).

3.4 | Reproductive parameters

Reproductive allotment (GSI) was highest in ALR females but this difference was statistically significant only compared to the SAK group (LME, pairwise estimate [se] 11.73 [3.20], $t_{16} = 3.66$, $P = 0.022$; Figure 3a; other comparisons $P > 0.214$; Supporting Information S28). Absolute fecundity was four times higher in BLW and ALR females than in SAK females ($P < 0.002$; Figure 3b and Supporting Information S29). Other comparisons were not statistically significant ($P > 0.088$; Supporting Information S29). Fecundity was not dependent on diet (Negative Binomial GLMM, $\chi^2_5 = 9.856$, $P = 0.079$) when corrected for female body size ($\chi^2_1 = 11.68$, $P < 0.001$; Supporting Information S30). Fertilization rate in the BLW group was nearly twice that of the SAK and SKR groups (binomial GLMM, $P = 0.003$; Supporting Information S31 and Figure 3c). Fertilization rate tended to be 18% higher in the BLW than the ALR ($P = 0.070$) and COP groups ($P = 0.086$; Figure 3c).

There were apparent relationships between fatty acid and amino acid composition and reproductive output. *N. furzeri* showed a trend towards increasing supply of dietary proline (functional non-EAA) and phenylalanine (aromatic EAA). All the other amino acids were present in optimal amounts in all the artificial diets (Supporting Information S32). The ratio of aromatic amino acids in the diet (phenyalanine to tyrosine) also appeared to be important. Subjecting killifish to a consistent nutrition history especially high in these amino acids (as in ALR, BLW) rendered a good fertilization rate and high embryo survival (Figure 3c,d). Histidine limitation in SAK and SKR could have also affected reproduction/fecundity, as body weight corresponded closely to the histidine content of the diet (Supporting Information S32). *N. furzeri* seem to fare well under low levels of sulphur-containing amino acids (as in BLW; Figure 3). On the other hand, a higher presence of some dietary PUFAs, such as α-linolenic acid (C18:3n-3) and arachidonic acid (C20:4n-6), in the SKR group was concomitant with relatively poor fertilization and embryo survivability rates (Figure 3 and Supporting Information S8 and S32), although somatic growth on this diet was favourable.

The highest embryo survival (31 dpf) was in the BLW and ALR groups, which showed significantly lower embryo mortality than the SKR group (coxme, pairwise comparison ratio [se], BLW-SKR 0.54 [0.12], $z = 2.82$, $P = 0.039$; ALR-SKR 0.55 [0.12], $z = 2.74$, $P = 0.049$; Figure 3d). Embryo mortality did not differ among the other diets ($P > 0.282$; Supporting Information S33). The SAK group showed a markedly higher proportion of fast-developing embryos after 31 dpf

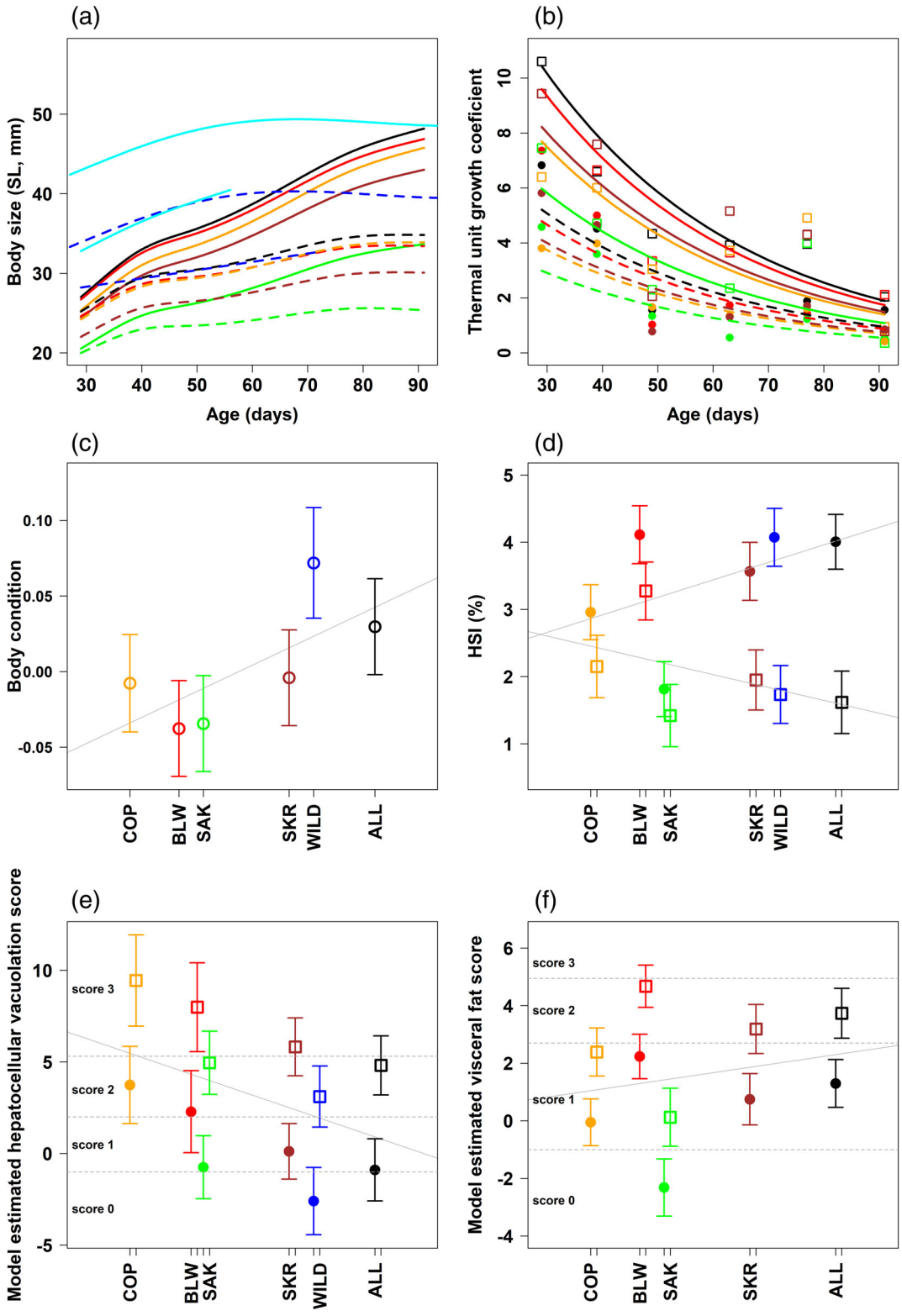

FIGURE 2 Legend on next page.

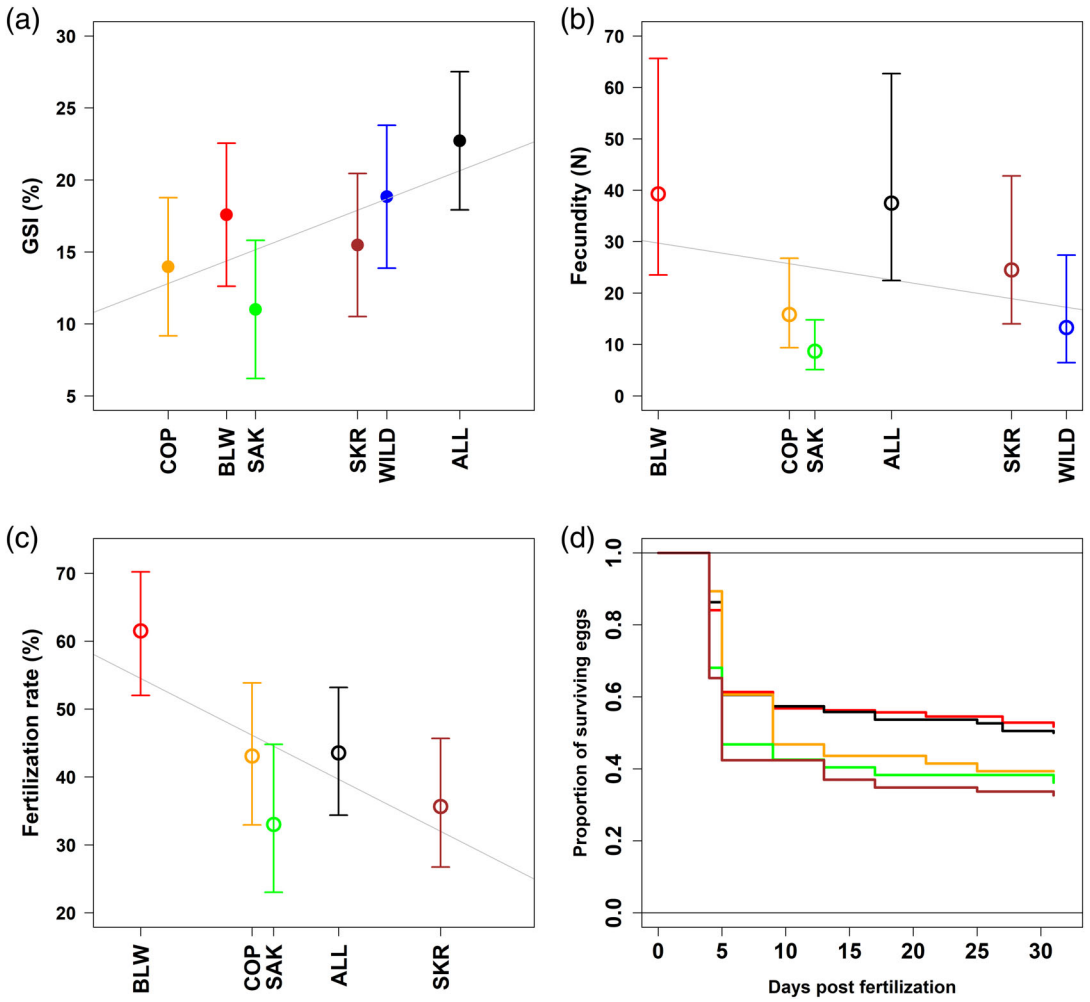

FIGURE 3 Reproductive parameters affected by diet. (a) Gonado-somatic index (GSI). The x axis is protein concentration of diet. GSI appears to increase with dietary protein concentration. Means and error bars (95% confidence intervals) are linear mixed effect (LME) estimates. (b) Diet-dependent absolute fecundity (negative binomial GLMM) uncorrected for body size. The x axis is diet lipid concentration, not protein, as lipids affects gamete quality. (c) Fertilization rate (binomial GLMM). The x axis is lipid concentration (Ruohonen et al., 2007), which appears to decrease with fertilization rate. (d) Egg survival to 31 dpf visualized in a Kaplan–Meier plot. (——) Aller Infa; (——) bloodworms; (——) Coppens Orange; (——) SAK MIX; (——) Skretting Vitalis. Analysis based on Cox proportional hazard mixed model. ALR, Aller Infa; BLW, bloodworms; COP, Coppens Orange; SAK, SAK MIX; SKR, Skretting vitalis; WILD, wild fish

FIGURE 2 Effect of diets on somatic parameters. (a) Age-dependent body size (standard length) of adults (age > 29 days). (——) ALR M; (——) BLW M; (——) COP M; (——) SAK M; (——) SKR M; (——) WILD M; (– –) ALR F; (– –) BLW F; (– –) COP F; (– –) SAK F; (– –) SKR F; (– –) WILD F. Curves produced from Gaussian generalized additive models. Curves for wild fish are presented only for visual comparison and were not included in statistical comparison. Confidence intervals (CIs) are not visualized to allow clear visualization of average trend (lines), confidence intervals may be found in Supporting Information S14. (b) Thermal-unit growth coefficient (TGC). (—□—) ALR M; (—□—) BLW M; (—□—) COP M; (—□—) SAK M; (—□—) SKR M; (-●-) ALR F; (-●-) BLW F; (-●-) COP F; (-●-) SAK F; (-●-) SKR F. Points are computed values at the end of the age interval for which they are computed. Curve produced from linear model, back-transformed log-TGC. (c) Diet-dependent body condition from length–weight residuals. (d) Hepato-somatic index with apparent sex-specific response to diet. (□) males; (●) females. (e) Diet-specific hepatocellular vacuolation. (□) male; (●) female. (f) Diet-dependent visceral fat score. Horizontal dashed grey lines in (e) and (f) delimit the band in which each score occurs (shown inside y axis). (□) male; (●) female. The x axis in (c)–(f) is the protein concentration of diets (Ruohonen et al., 2007). The grey line in (c)–(f) is the regression trend of crude protein and average values of metrics. Error bars in (c)–(f) are the model estimated 95% confidence intervals. Means and 95% CI in (c) and (d) from linear mixed effect model. Means and 95% CI in (e) and (f) from ordinal GAM. ALR, Aller Infa; BLW, bloodworms; COP, Coppens Orange; SAK, SAK MIX; WILD, wild fish; M, male; F, female

than the BLW group (binomial GLMM, pairwise odds, ratio [se], 0.10 [0.06], $z = 3.97$, $P = 0.001$), and tended to be higher than SKR ($P = 0.055$) and the ALR group ($P = 0.058$; Supporting Information S34 and S35). Other comparisons were not significant ($P > 0.164$; Supporting Information S34). An overview of all results is reported in Supporting Information S36.

4 | DISCUSSION

Unlike in zebrafish' (Watts & D'Abramo, 2021), which have been used in laboratory research for decades, there are considerable gaps in our knowledge concerning the nutrition of captive *N. furzeri*. This limits fuller use of this animal model in biomedical research, including ageing-related and toxicological studies. Here we show that *N. furzeri* target high-protein food in the wild and perform best when fed protein-rich animal-based feeds. Bloodworm biases body condition and liver size compared to wild fish and thus does not represent an ideal diet for laboratory *N. furzeri*. In addition to demonstrating the importance of proximate composition (macronutrients) in the artificial diets, we also provide indirect insights into the role of protein- and lipid-specific composition in *N. furzeri* performance.

4.1 | Nutritional parameters

Diets with similar crude protein content but different amino acids will have different effects on fish performance (Dabrowski & Guderley, 2003; Tacon & Cowey, 1985). In most artificial diets for fishes, lysine and methionine are limiting EAAs and their insufficient levels may retard growth (Dabrowski & Guderley, 2003). Accordingly, the ideal protein concept (Rollin *et al*., 2003) is build either around lysine (present study and Kaushik & Seiliez (2010)) or methionine (Turchini *et al*., 2019). *N. furzeri* appears to have high demand for histidine (or its acetylated form; Yamada *et al*., 2009), which is common among small-sized, short-cycle breeding carnivorous fish (*e.g*., anabantids) and some African cichlids (Moro *et al*., 2020; Yamada *et al*., 2009). Proline is an essential amino acid for both juvenile and adult fish under certain circumstances (Wu *et al*., 2011). *N. furzeri* showed positive, shared dependency on proline and phenylalanine for growth and reproduction. Proline contributes to good-quality sperm (Butts *et al*., 2020; Lahnsteiner, 2009), which may explain the high fertilization rate observed in the BLW group as this diet supplied the highest level of proline. In fish, phenylalanine and tyrosine are the key precursors of hormones and neurotransmitters involved in growth, stress response (Salamanca *et al*., 2021) and fish pigmentation (Cheng, 2008; Nüsslein-Volhard & Singh, 2017). The conspicuous pigmentation of the *N. furzeri* body and its short, sexually active (and likely hormonally dynamic) life cycle seem to be drivers for increased phenylalanine requirements.

The mentioned amino acid results should be taken as preliminary because nutrients interact each other (Raubenheimer & Simpson, 2019) and are here inferred from diets made of practical but unrefined ingredients. In addition, levels of amino acids which exceed the requirement may not elicit a positive physiological response and such oversaturation would not be detected in our design. The limiting amino acids appear more important (Heger & Frydrych, 2019).

Protein digestibility from insect larvae may be impaired by chitin (Marono *et al*., 2015), which may be the cause of the delayed peak in solid waste production and the poorest FCR in fish fed the BLW diet. Additionally, excess P:N ratio and/or high carbohydrate content in SAK and COP might be also linked to reduced digestibility and consequently poorer FCR than for ALR and SKR. Phosphorus locked in bone (animal origin) or phytate complex (plant origin) is generally poorly digested by fish (Hua & Bureau, 2010) and carnivorous fishes like *N. furzeri* do not digest vegetable-origin carbohydrates efficiently (Hemre *et al*., 2002).

The satiation of fish was not dependent on the amount of food consumed but probably on targeting daily protein, amino acid or energy requirements (Raubenheimer & Simpson, 2019; Stephens & Krebs, 1971). Fish satiation with dry feed was reached at a quarter of the amount required for bloodworms, which contains 80% water. The physical properties of food do not explain the differences between the amount of bloodworms and dry feed consumed because in the wild, fish consume a diet comparable in water content to bloodworms but they ingest only about 1.5%–2.5% of their body mass. Excessive overfeeding (15%–25% of body mass) with bloodworms in captivity is likely the consequence of time-constrained access to food and may have considerable negative health impacts. Differences in physical properties between bloodworm and dry feeds were reflected in the manner in which the food was consumed. Killifish accepted dry feed mostly from the water column and the water surface. When the pellets sank to the bottom they also consumed those. Bloodworms were mostly consumed from the bottom because they sink quickly. All food in all treatments was consumed within 2–3 min and the observed performance differences between pelleted-fed fish and bloodworm-fed fish are unlikely to be related to feed-dependent nutrient leakage prior to food consumption.

There were apparent sex-specific responses to the diets, which may be the consequence of different physiological requirements for growth and reproduction (Parker, 1992; Trivers, 1972). Female killifish are small but have exceptionally high reproductive costs as they maintain large gonads and produce large costly gametes daily (Trivers, 1972; Vrtílek & Reichard, 2015). Female reproductive allotment is so large that despite apparent differences in body shape between sexes (deeper males but rounded females) they have a similar length–weight relationship. Wild females have a slightly longer gut and consume twice as much food relative to their body mass as males, which may contribute to covering their excessive reproductive costs. In contrast, males grow larger as a consequence of competition for access to reproduction (Cellerino *et al*., 2016; Parker, 1992). The competitive nature of males may explain why they consumed twice as much food as females in communal tanks in captivity (Ward *et al*., 2006). Captive male overfeeding explains their higher visceral fat deposition compared to females. Sex-specific nutritional requirements should be

taken into account in the future development of standardized laboratory diet.

4.2 | Somatic parameters and growth

Well-fed and overfed fish have large livers (Chellappa *et al*., 1995; Ruohonen *et al*., 2007) and active reproduction also increases liver size (Evans, 1998). Wild females had larger livers than males likely due to their intensive daily reproductive output (Vrtílek & Reichard, 2016; Žák *et al*., 2019). This natural female–male difference in liver size was found also when fish were fed carnivorous diets (ALR, SKR). In contrast, there was a negligible difference in liver size in the bloodworm and omnivorous diet groups. The bloodworm diet resulted in male liver enlargement probably as a consequence of overfeeding. Bloodworm-induced liver enlargement is concerning as the liver is frequently investigated in studies of age-related changes (Baumgart *et al*., 2015; Di Cicco *et al*., 2011; Muck *et al*., 2018). SAK-fed females had smaller livers as a result of malnutrition and COP contributed to female liver enlargement probably as a consequence of high lipid ingestion to fulfil the protein requirements of a low-protein feed ('protein leverage theory'; Raubenheimer & Simpson, 2019).

Fish growth depends on food quantity (Vrtílek & Reichard, 2015), the source of protein in feed (Smith *et al*., 2013) and the protein amino acid profile (Tacon & Cowey, 1985). Reducing the feeding regime from twice to once per day was reflected in our study by a growth decrease at 39–49 dph. In contrast to many captive-bred fish species, growth in captive *N. furzeri* is slower than in the wild even under intensive effort (Blažek *et al*., 2013). The feeding regime or amino acid composition of feed for juvenile fish thus may be inadequate (Dabrowski, 1986; Tacon & Cowey, 1985). Comparable growth in aquaculture feeds is likely a consequence of the appropriate amino acid composition of proteins, in contrast to SAK, which deviated considerably from an ideal protein composition. Although growth is an important indicator of performance, it does not necessarily reflect the health status of fish (Watts *et al*., 2016).

4.3 | Health indicators

Health indicators were affected in a diet-specific manner. Overall health indices were worst in the SAK group, where body condition was low, liver size was decreased in females and deformities were prevalent. The bloodworm group showed mediocre results with low body condition, heavily vacuolated and enlarged livers but no bone deformities or decreased survival. The remaining artificial diets resulted in intermediate health performance and bone deformities were present in a few fish. Higher incidence of bone deformities in dry feeds compared to bloodworm-fed and wild fish may be caused by micronutrient oxidation during the repelletization process or by micronutrient/trace element deficiency in the diet (Lall & Lewis-McCrea, 2007). In our previous study, we used dry feed without repelletization and no bone deformities were observed (Žák *et al*., 2020). The bone deformities seen in the dry feed groups were mostly small jaw malformations, which only rarely negatively affected animals. Survival was similar among the experimental diets and comparable with other studies (*e.g*., Blažek *et al*., 2017). It is difficult to determine the underlying mechanisms responsible for the different condition indices without a knowledge of the proximate composition of fish bodies, but the lower body condition of captive fish fed live food than those in the wild is unexpected. However, a similar finding was also reported in zebrafish (Fowler *et al*., 2019). The superior body condition of wild fish may be related to their fast growth and higher muscle build-up (also supported by the condition index calculated from eviscerated body mass). Low body condition in the SAK group is apparently related to malnutrition. Histological examination did not reveal any accumulation of crystals in the kidneys or any degenerative or proliferative changes in the intestine or liver. Liver histology revealed decreasing liver vacuolation with increasing protein in feed, meaning that proteins were readily processed for energy metabolism and there was no need to maintain excessive energy stores in livers (Evans, 1998; Ruohonen *et al*., 2007). Health indicators on a molecular level should be investigated in the future (Williams & Watts, 2019).

4.4 | Reproduction

Diet affected reproductive parameters. The lowest reproductive allotment in the SAK group again stems from malnutrition as females invested into somatic maintenance rather than into reproduction (Vrtílek & Reichard, 2015). Absolute fecundity was similar in the BLW and ALR groups but lower in other diets. This difference in fecundity was a consequence of the diet-dependent body size of females because analysis corrected for body size revealed no significant differences in fecundity. Our results suggest that the dry feeds show satisfactory results for egg production but the lower fertilization rate should be improved in the future.

Fertilization rate appeared to be negatively correlated to total lipid content in diet. This contradicts the assumption that high lipid content is necessary for good reproductive function in *N. furzeri* (Tozzini & Cellerino, 2020) but accords with the general nutritional requirements of warm-water fish species (NRC, 1977). From the lipid composition perspective, the present study hinted at some fatty acids that were previously identified as contributors to fertility problems in fishes and terrestrial vertebrates if present in higher amounts. For example, the relatively high amounts of α-linolenic acid (ALA) and arachidonic acid (ARA) in SKR feed could be linked with poor fertilization rate and lower embryo survival (ALA: Jungheim *et al*., 2011; Marei *et al*., 2010; ARA: Ljubobratović *et al*., 2020; Surai *et al*., 2000). The generally lower fertilization rate in the dry feed groups compared to the BLW group is commonly found when live feeds and dry feeds are compared (Mandal *et al*., 2012; Markovich *et al*., 2007).

Developing embryos rely solely on resources provided by the mother during oogenesis and thus maternal diet may affect embryo development (Riddle & Hu, 2021). We found a significantly higher proportion of fast-developing embryos in the SAK group, where females were considerably malnourished and least fecund. Longer developing

embryos have a reduced yolk size at hatching, which indicates how costly the extension of embryo development is (Polačik *et al*., 2014). Thus, embryos with depleted or low-quality resources may develop faster to reduce the costs of extended development. Diets which contributed to good performance in parents also supported good embryo survival, which supports the importance of good parental nutrition for embryo development (Riddle & Hu, 2021). The performance of offspring produced by parents fed different diets should be investigated in the future.

Creating a single laboratory diet which would support optimal growth, reproduction and body maintenance is unrealistic. The desired goal of experimental fish husbandry is the replicable composition of diet and its good quality with regard to fish health and performance. The commercial feeds currently available seem to fulfil this goal despite the disadvantage of a closed formula. The finding that a starter feed (Aller Infa) is a practical replacement for bloodworms for *N. furzeri* is in accordance with a previous study where starter feed for salmonids was used (Žák *et al*., 2020). Starter feeds are usually rich in phospholipids, proteins and free amino acids (Dabrowski, 1986) and the good performance of *N. furzeri* on these diets probably stems from their high dietary requirement for those nutrients. It should be noted that this and the previous study (Žák *et al*., 2020) were conducted on juveniles and young adults, and diet composition requirements may change later in life. The apparent variability in *N. furzeri* performance among different diets under an identical feeding protocol confirms that the reproducibility of results is limited when different diets are used.

5 | CONCLUSIONS

The first comparison of four different dry feeds on performance of *N. furzeri* laboratory fish with increasing importance in ageing and toxicology research clearly demonstrates that this species is not reluctant to accept dry feed, as suggested hitherto. Bloodworm may therefore be replaced by dry feed in the captive breeding of this species, which would contribute to husbandry standardization among different laboratories. This study showed multiple detrimental effects of using bloodworms in *N. furzeri* husbandry and these (such as the overfeeding, enlargement of liver and low body condition) should be taken into consideration when planning experimental laboratory studies. Starter dry feeds with high protein content contribute to satisfactory performance, including fecundity, and thus are suitable bloodworm replacements. However, the decreased fertilization rate of pelleted diet-fed fish should be improved in the future. The findings presented here are promising for the further development and use of purified diets consisting of more refined and chemically defined ingredients.

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COMPETING INTERESTS

The authors have no relevant financial or nonfinancial interests to disclose.

AUTHOR CONTRIBUTIONS

J.Ž.: conceptualization, data curation (fish performance and proximate composition of feeds), formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, visualization, writing original draft. K.R.: conceptualization, data curation (fatty and amino acids), methodology, writing – review and editing. I.D.: investigation (histology), writing – review and editing. J.M.: conceptualization, data curation (fatty and amino acids), funding acquisition, methodology, writing – review and editing. M.R.: project administration, resources, funding acquisition, supervision, writing – review and editing.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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SUPPORTING INFORMATION

Starter feed for carnivorous species as a practical replacement of bloodworms for a vertebrate model organism in ageing, *Nothobranchius furzeri*

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SUPPORTING INFORMATION S1
FISH HUSBANDRY

Embryos from the wild derived population MZCS 222 of *Nothobranchius furzeri* (Cellerino et al., 2016) were disinfected by a 15 min bath in 0.005 % peracetic acid two weeks before hatching to prevent disease introduction and then incubated at 30 °C for two weeks in autoclaved peat. Embryos were hatched on 26 October 2020 by pouring 17° C water onto the peat substrate with ready-to-hatch eggs in a 5L glass aquarium. At 3 days post hatching (dph), killifish were moved to 60L aquaria. Fish were transferred from single 60L aquarium into ten 9L aquaria in a recirculating system (RS) at 18 dph. Water parameters in RS were kept constant during the whole experimental period (reverse osmosis water, conductivity 2000 $\mu S \times cm^{-2}$ increased with Cichlid Lake Salt (Seachem, USA) and kitchen salt in a 1:4 ratio, compartment inflow 1.25 $L \times min^{-1}$, temperature: 26.8 ± 0.36 °C (mean ± SD), oxygen: 9-10 $mg \times l^{-1}$, pH: 7.0-7.2, total ammonia < 0.3 $mg \times l^{-1}$, nitrite and nitrate < 3 $mg \times l^{-1}$, tested with eSHa Aquatest (eSHa labs, Netherlands)). Water temperature in RS was monitored by two temperature loggers (HOBO UA-002–64, Onset Computer, Bourne, MA, USA) with a 4 hour logging interval. The bottom was siphoned daily and 25 % volume of RS water was replaced every 10 days. The light regime was 14L:10D (L: 6:00-20:00).
Initial fish density in RS was set to 20 individuals per 9L aquarium which improves dry feed acceptance via social facilitation (Stoner and Ottmar, 2004; Žák et al., 2020). Density was reduced to 10 individuals per aquarium at 21 dph when all fish were fully weaned onto experimental diets. This resulted in 4 replicates of mixed sex aquaria for each dietary treatment (N = 40 individuals per diet). The position of each aquarium for each diet was randomly distributed within the RS and kept constant during the experiment. Fish size was standardized across aquaria for each treatment by translocating fish every 2-5 days to reduce aggression and allow equal access to food for all individuals (Polačik et al., 2016). As fish grew, the density was reduced to 8 fish per 9L aquarium (N = 32 per diet) at 53 dph and this density was kept until experiment termination at 91 days.

SUPPORTING INFORMATION S2: Ingredient lists of pelleted feeds used.

Aller Infa 0.4 mm: fish meal, wheat gluten, krill meal, wheat, lecithin,
Batch number: 58000540

Skretting Vitalis 2.5 mm: fish meal, squid meal, crustacea meal, wheat gluten, manioc starch, fish solubles, alfalfa protein concentrate, soya protein concentrate, lecithin, fish oil, dicalcium phosphsate, algae meal, vitamins, minerals, astaxanthin. Batch number: 7349612

Coppens Orange 3 mm: wheat, fish meal, sunflower meal, wheat gluten, soya protein concentrate, lecithin, monocalcium phosphate, krill meal, yeast products, pepper, inulin, algae (*Schizochytrium limacinum*), binder (1m558i Bentonite 1000mg/kg). Batch number: 029588

SAK Mix: fish meal, wheat flour, rice flour, shrimp meal, squid meal, krill meal, *Tenebrio molitor* meal, rye flour, oatmeal, soya meal, corn gluten, yeast products, salmon oil, potato flakes, pea powder, algae (Chlorella sp., *Ascophyllum nodosum*), mix of vitamins, mix of medicinal herbs, zeolite, *Moringa oleifera* leaf powder, lysin, herbs (*Taraxacum* sext. *ruderalia*, *Urtica dioica)*, beetroot powder, Shiitake, Spirulina, pentasodium triphosphate, inulin, threonine, betain, astaxanthin, red pepper colorant, betaglucan, canthaxanthin, methionine, betacaroten 10 %. Batch number: NA

Bloodworm: 100 % frozen chironomid larvae, supplier: Grýgera, nakrmryby.cz, Batch number: NA

SUPPORTING INFORMATION S3: Proximate composition reported by producers of commercially available diets.

	Aller Infa 0.4 mm N	Skretting Vitalis 2.5 mm N	Coppens Orange 3 mm N	SAK Mix 0.7-1 mm N
Crude protein (%)	64.00	59.00	45.00	54.00
Crude fat (%)	8.00	11.00	7.00	9.38
Moisture (%)	-	-	-	-
Carbohydrates (NFE, %)	8.90	-	-	-
Ash (%)	12.10	11.50	8.90	10.51
Fibre (%)	1.00	0.30	2.80	2.51
Total phosphorus (%)	1.40	1.30	1.26	1.63
Calcium (%)	2.30	-	1.60	2.27
Sodium (%)	0.70	-	0.30	-

SUPPORTING INFORMATION S4:

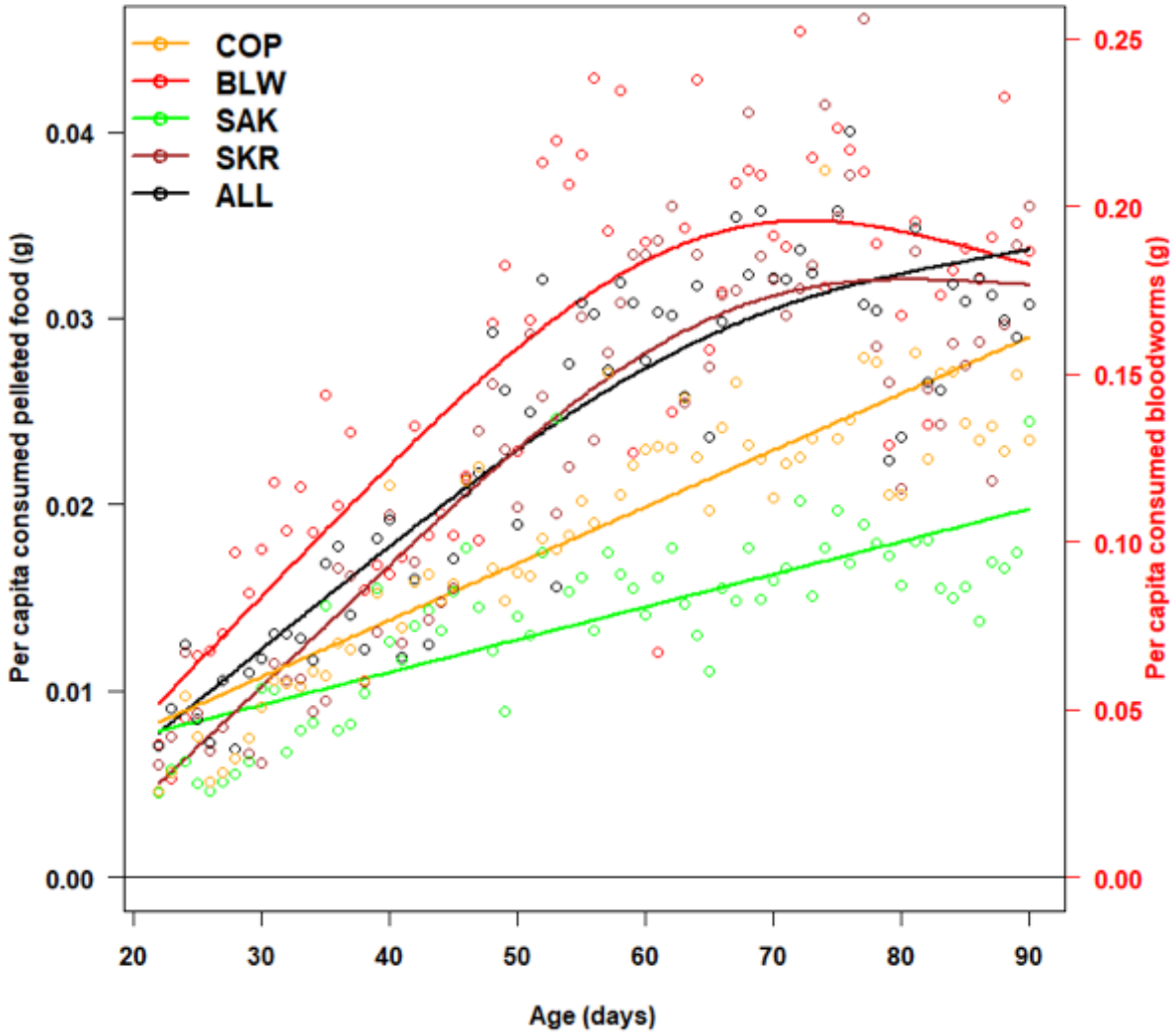
COLD REPELLETIZATION PROCEDURE

Cold repelletization of dry feeds was performed to reduce feed density variance (Table 1) caused by the specific extrusion process of each feed. The cold repelletization process consisted of grinding each feed to fine particles with an electric grinder and adding 300 ml water per 1 kg feed to make a paste dough. The dough was processed in a noodle maker into 2 mm x 5-7 mm pellets. The pellets were fan-dried at room temperature for 8 hours and then oven dried at 45 °C for 48 hours. The dried pellets were ground in a mincer and sieved through stainless steel sieves (20 cm diameter, de Buyer, France, https://www.debuyer.com/) to pellet sizes of 0.4-0.5 mm, 0.6-1.4 mm and 1.5-2 mm. The finest dust was removed by sieving through 0.3 mm mesh. The stock package of feed was refrigerated at 4-6 °C and experimental batches of feed kept at room temperature and replaced every two weeks. This storage protocol safely reduced any fungal development in the repelletized feed. Proximate composition of repelletized diets is shown in Table 1.

SUPPORTING INFORMATION S5: Macronutrient proximate composition of dietary items found in wild fish digestive tracts used for computation of target macronutrient composition of the best diet for experimental *Nothobranchius furzeri*. Proportions are averages from corresponding references and are not corrected for chitin.

Dietary item	Average protein (%)	Average Lipid (%)	Sources of macronutrient composition
Chironomidae larvae	52.17	4.05	Present study, (De La Noüe and Choubert, 1985; Roy et al., 2020; Žák et al., 2020)
Zooplankton	62.85	12.20	(Mischke et al., 2003; Riccardi and Mangoni, 1999; Roy et al., 2020)
Odonata larvae	62.94	3.20	(González et al., 2020)
Insect meal (terrestrial insect, aquatic Copepoda)	53.23	20.04	(Bamidele et al., 2021; Hlongwane et al., 2020; IAFFD, 2020; Razeng and Watson, 2015; Turek et al., 2020)
Ephemeroptera larvae	58.98	14.80	(González et al., 2020)
Culicidae	42.20	16.10	(Habashy and Daba, 2006)
Notonectidae (backswimmers)	74.87	15.37	(Das et al., 2011)
Amphibia (tadpoles)	47.96	16.46	(Afonso et al., 2017; Dierenfeld et al., 2002; Sogbesan and Ugwumba, 2008)

SUPPORTING INFORMATION S6:



SUPPORTING INFORMATION S6: Per capita Absolute amount of food consumed per capita during the whole course of the experiment. Values are not corrected for body mass. The morning feeding was used on days when fish were fed more than once. Points = observations for each day. Curves are produced by Gaussian GAM. No error bars were used for better clarity of the plot. Note the different y-axis for the bloodworm group on the right-hand side.

SUPPORTING INFORMATION S7: Overview of models used for data analysis. N = number of all measurements in the analysis, NRF = number of levels of random factors, Aquarium_site is combined factor for Aquarium ID from dietetic experiment and site in the wild. aquarium_samp is combined factor for aquarium ID from dietetic study and repeated sampling points at a single site in wild. HIS = hepato-somatic index, FCR = feed conversion ratio, TGC = thermal growth coefficient, SL = standard length, dph = days post hatching

	Model structure	Sample size
Age dependent body mass of adults	gam(log(bm)~s(age,k=6)+s(age,by=diet,k=6)+s(age,by=sex,k=6)+diet*sex+s(aquarium,bs="re"))	N = 1079; NRF = 20
Age dependent SL of adults	gam(log(SL)~s(age,k=6)+s(age,by=diet,k=6)+s(age,by=sex,k=6)+diet*sex+s(aquarium,bs="re"))	N = 1069; NRF = 20
Initial body mass (19 dph)	lmer(log(bm)~diet+(1|aquarium))	N = 198; NRF = 10
Juvenile growth in bm (29 dph)	lmer(log(bm)~diet+sex+(1|aquarium))	N = 200; NRF = 20
Body condition	lm(residuals_from_L_W~diet+sex)	N = 171
Growth (TGC)	lm(log(tgc)~age+diet+sex)	N = 60
Gut length	lmer(rel_gut_length~diet+sex+gut_fullness+(1|aquarium_samp))	N = 144; NRF = 24
HSI	lmer(HSI~diet*sex+ (1|aquarium_site))	N = 143; NRF = 24
Hepatocellular vacuolation	gam(vac_score~diet+sex+s(aquarium_site,bs="re"),ocat(R=4))	N = 46; NRF = 24
Morphological type of vacuolation	chisq.test(diet,vacuolation.type)	N=46
Liver storage fat	lmer(asin(sqrt(liver_fat))~diet+sex+(1|aquarium))	N = 39; NRF = 20
Liver water content	lmer(asin(sqrt(liver_water))~diet+sex+(1|aquarium))	N = 39, NRF = 20
Visceral fat score	gam(vf~diet+sex+s(aquarium,bs="re"),ocat(R=4))	N = 141; NRF = 20
Fecundity	glmer.nb(totN_eggs~diet+SL+(1|aquarium))	N = 96; NRF = 12
Fertilization rate	glmer(cbind(fertilized,unfertilized)~diet+(1|aquarium))	N = 80; NRF =10
Egg survival	coxme(Surv(dpf,death)~diet+(1|plate))	N = 599; NRF = 10
Egg development	glmer(stage~diet+(1|plate),binomial)	N = 275; NRF = 10
Age and diet dependent amount of food consumed	lmp(proportion of consumed food to bm ~age+diet)	N = 30
Sex dependent amount of food consumed	lmer(log(consumed_per_bm)~diet*sex+(1|aquarium_samp))	N = 100; NRF = 25
FCR	lm(log(fcr)~age+diet)	N = 25

SUPPORTING INFORMATION S8: Ideal protein concept for turquoise killifish *Nothobranchius furzeri* and comparison of protein quality of the experimental diets. All values are expressed in % lysine. Red cells indicate under-supplied AAs in the dietary protein profile (if dietary AAs negatively deviate from ideal protein composition for *N. furzeri* by -10% or less). Green cells indicate over-supplied AAs in the dietary protein profile (if AAs positively deviate from ideal protein composition for *N. furzeri* by +10% or more).

Amino acids	Ideal protein*	ALL	SAK	COP	SKR	BLW
Met	52.7	44.7	34.3	55.4	50.3	32.1
Thr	59.1	67.6	59.5	86.9	78.3	88.6
Asp	131.4	150.5	130.2	192.5	168.4	237.1
Ser	60.4	83.5	65.5	114.3	87.7	99.5
Glut	221.7	379.5	185.1	465.4	272.6	316.8
Gly	115.4	98.1	90.1	120.4	117.1	84.2
Ala	106.8	93.6	83.5	111.5	108.3	171.3
Tyr	50.3	67.1	46.7	74.8	51.2	73.8
Val	72.3	80.7	64.1	106.7	85.6	95.0
Phe	61.6	68.8	46.6	91.7	59.2	121.3
Ileu	58.1	71.1	54.9	83.1	70.1	110.4
Leu	115.2	133.4	104.0	169.4	138.0	134.2
Hist	47.6	41.7	29.2	58.8	34.8	61.9
Arg	73.6	73.8	76.4	113.9	93.7	94.6
Cys	16.8	23.5	14.3	37.2	22.2	0.0
Pro	14.6	12.5	5.9	19.0	14.2	34.0
Tryp	4.9	4.1	3.1	6.0	4.0	22.4
Average		☆13.7	☆-9.9	★37.9	☆11.4	★29.2

*Ideal protein concept for killifish: all amino acids expressed as % of lysine. Lysine= highest EAA in killifish body protein

SUPPORTING INFORMATION S9

SUPPORTING INFORMATION S9A: Fatty acid composition of diets used for comparison of performance in *Nothobranchius furzeri*. Values represent back-calculated relative proportion (%) of specific fatty acid in total content of the diet. Fat content* is determined from wet matter (not dry matter) thus it differs slightly from the results in Table 1. # The bloodworm used for analysis came from a different batch than that used for the feeding experiment.

Fatty acid	Aller INFA	SAK MIX	Coppens orange	Skretting VITALIS	Bloodworms#
Fat content*	9.4	6.7	4.0	14.4	4.5
C14:0	0.41	0.32	0.09	0.72	0.26
C14:1	0.01	0.01	0.00	0.02	0.05
C16:0	1.70	1.24	0.53	2.90	0.97
C16:1	0.39	0.31	0.11	0.61	0.49
C18:0	0.24	0.22	0.09	0.5	0.34
C18:1n-9	2.31	1.39	1.34	2.35	0.58
C18:1n-7	0.41	0.26	0.11	0.48	0.46
C18:2n-6	1.32	0.91	1.06	2.26	0.54
C18:3n-3	0.23	0.21	0.19	0.53	0.11
C20:0	0.02	0.01	0.01	0.04	0.05
C20:1n-9	0.39	0.35	0.03	0.50	0.01
C20:2n-6	0.01	0.02	0.01	0.03	NA
C20:4n-6	0.05	0.04	0.01	0.12	0.00
C20:3n-3	0.01	0.01	NA	0.02	0.03
C22:0	0.01	0.01	0.01	0.03	0.00
C22:1n-9	0.07	0.05	0.01	0.06	NA
C20:5n-3	0.74	0.50	0.18	1.10	0.36
C24:0	0.01	0.01	0.01	0.02	NA
C24:1n-9	0.06	0.05	0.01	0.08	NA
C22:5n-3	0.05	0.06	0.02	0.13	NA
C22:6n-3	0.94	0.68	0.20	1.84	0.00

SUPPORTING INFORMATION S9B: Summary of the fatty acid composition of diets used for performance comparison of *Nothobranchius furzeri*.

	Aller INFA	SAK MIX	Coppens orange	Skretting VITALIS	Bloodworms
SFA	2.39	1.81	0.74	4.27	1.76
MUFA	3.64	2.41	1.60	4.10	1.65
PUFA	3.36	2.43	1.67	6.01	1.08
n-3	1.97	1.46	0.59	3.61	0.51
n-6	1.38	0.97	1.08	2.40	0.56
n6/n3 ratio	0.70	0.66	1.84	0.66	1.11
n3/n6 ratio	1.43	1.51	0.54	1.50	0.90
n-3 HUFA	1.74	1.26	0.40	3.08	0.40
EPA + DHA	1.68	1.19	0.37	2.94	0.37
PUFA/ MUFA Ratio	0.92	1.01	1.04	1.47	0.65

SUPPORTING INFORMATION S10: Statistical results of diet specific food conversion ratio (FCR). Gaussian Linear Model.

```
##
## Call:
## lm(formula = log(fcr) ~ age + diet, data = fcr_s2)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.33982 -0.13680 -0.02501  0.15697  0.64703
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) -0.748077   0.187828  -3.983 0.000797 ***
## age          0.018562   0.002462   7.539  4.0e-07 ***
## dietBLW      1.791493   0.145617  12.303  1.7e-10 ***
## dietCOP      0.061161   0.145617   0.420 0.679188
## dietSAK      0.228305   0.145617   1.568 0.133422
## dietSKR      0.006933   0.145617   0.048 0.962522
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2302 on 19 degrees of freedom
## Multiple R-squared:  0.937,  Adjusted R-squared:  0.9204
## F-statistic: 56.52 on 5 and 19 DF,  p-value: 9.522e-11
```

```
## $emmeans
##  diet response    SE df lower.CL upper.CL
##  ALL      1.55 0.159 19     1.25     1.92
##  BLW      9.28 0.955 19     7.48    11.51
##  COP      1.64 0.169 19     1.33     2.04
##  SAK      1.94 0.200 19     1.57     2.41
##  SKR      1.56 0.160 19     1.26     1.93
##
## Confidence level used: 0.95
## Intervals are back-transformed from the log scale
##
## $contrasts
##  contrast  ratio     SE df t.ratio p.value
##  ALL / BLW 0.167 0.0243 19 -12.303 <.0001
##  ALL / COP 0.941 0.1370 19  -0.420 0.9929
##  ALL / SAK 0.796 0.1159 19  -1.568 0.5341
##  ALL / SKR 0.993 0.1446 19  -0.048 1.0000
##  BLW / COP 5.643 0.8216 19  11.883 <.0001
##  BLW / SAK 4.774 0.6952 19  10.735 <.0001
##  BLW / SKR 5.957 0.8674 19  12.255 <.0001
##  COP / SAK 0.846 0.1232 19  -1.148 0.7796
##  COP / SKR 1.056 0.1537 19   0.372 0.9955
##  SAK / SKR 1.248 0.1817 19   1.520 0.5626
##
## P value adjustment: tukey method for comparing a family of 5 estimates
## Tests are performed on the log scale
```

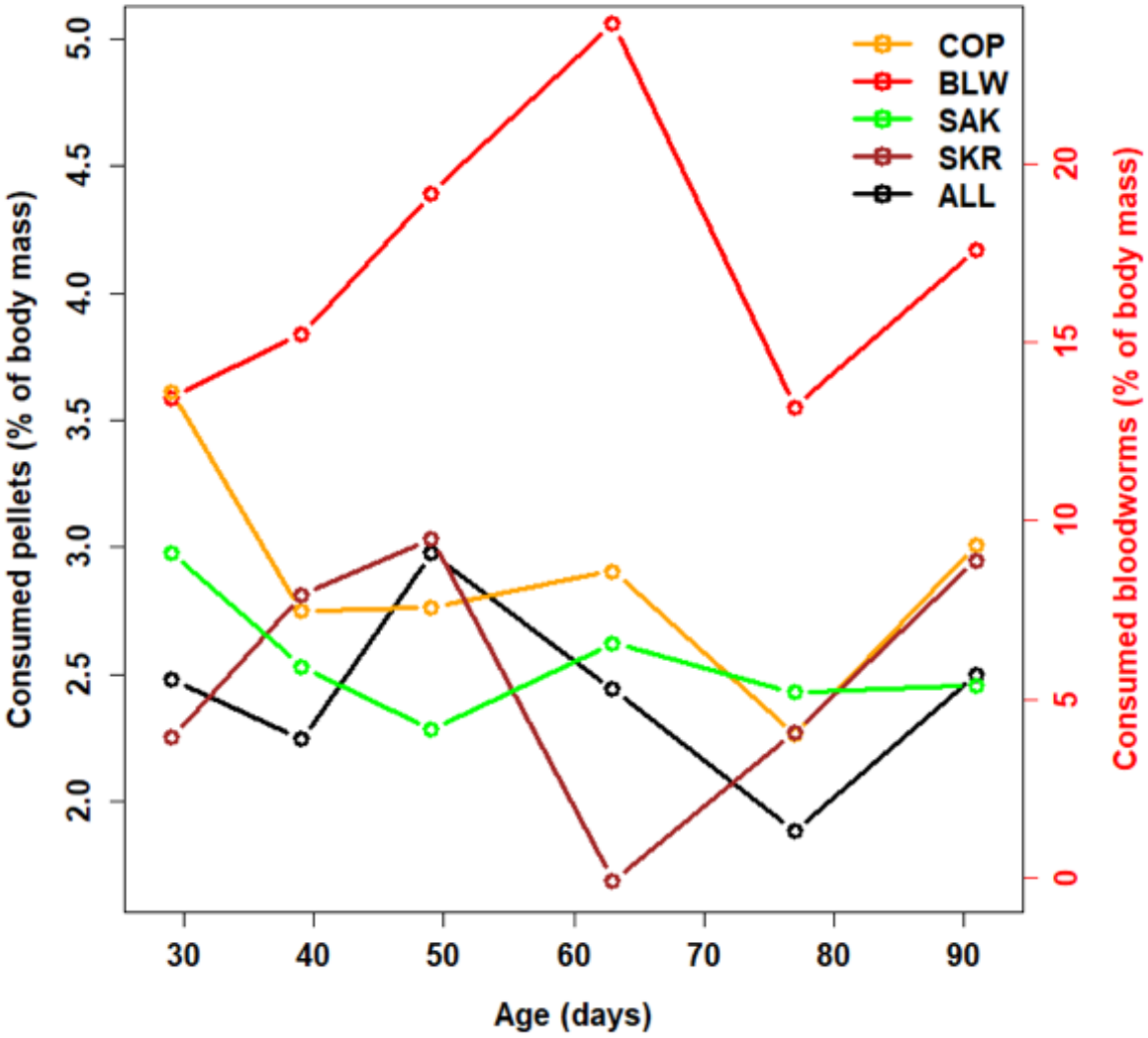
SUPPORTING INFORMATION S11A: Statistical results of sex specific amount of food consumed. Linear mixed effect model.

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: log(eaten_per_g) ~ sex * diet + (1 | tank)
##    Data: eater2
##
## REML criterion at convergence: 39.4
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.00215 -0.57908  0.04236  0.50814  2.40749
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  tank     (Intercept) 0.05333  0.2309
##  Residual             0.05310  0.2304
## Number of obs: 100, groups:  tank, 25
##
## Fixed effects:
##                Estimate Std. Error t value
## (Intercept)   -2.124213   0.182489 -11.640
## sexM           0.732943   0.258079   2.840
## dietALL       -1.469924   0.258079  -5.696
## dietCOP       -1.510271   0.258079  -5.852
## dietSAK       -1.597926   0.258079  -6.192
## dietSKR       -1.603576   0.258079  -6.214
## dietwild      -1.483489   0.221987  -6.683
## sexM:dietALL  -0.549276   0.364978  -1.505
## sexM:dietCOP  -0.081322   0.364978  -0.223
## sexM:dietSAK  -0.259826   0.364978  -0.712
## sexM:dietSKR  -0.008634   0.364978  -0.024
## sexM:dietwild -1.463997   0.277892  -5.268
##
## Correlation of Fixed Effects:
##             (Intr) sexM   ditALL ditCOP ditSAK ditSKR ditwld sM:ALL sM:COP
## sexM        -0.707
## dietALL     -0.707  0.500
## dietCOP     -0.707  0.500  0.500
## dietSAK     -0.707  0.500  0.500  0.500
## dietSKR     -0.707  0.500  0.500  0.500  0.500
## dietwild    -0.822  0.581  0.581  0.581  0.581  0.581
## sexM:ditALL  0.500 -0.707 -0.707 -0.354 -0.354 -0.354 -0.411
## sexM:ditCOP  0.500 -0.707 -0.354 -0.707 -0.354 -0.354 -0.411  0.500
## sexM:ditSAK  0.500 -0.707 -0.354 -0.354 -0.707 -0.354 -0.411  0.500  0.500
## sexM:ditSKR  0.500 -0.707 -0.354 -0.354 -0.354 -0.707 -0.411  0.500  0.500
## sexM:ditwld  0.657 -0.929 -0.464 -0.464 -0.464 -0.464 -0.626  0.657  0.657
##             sM:SAK sM:SKR
## sexM
## dietALL
## dietCOP
## dietSAK
## dietSKR
## dietwild
## sexM:ditALL
## sexM:ditCOP
## sexM:ditSAK
## sexM:ditSKR  0.500
## sexM:ditwld  0.657  0.657
```

SUPPORTING INFORMATION S11B: Statistical results of sex specific amount of food consumed. Pairwise contrasts

```
## $emmeans
## diet = BLW:
##  sex response      SE df lower.CL upper.CL
##  F     0.1195 0.02181 14   0.0808   0.1768
##  M     0.2488 0.04540 14   0.1682   0.3679
##
## diet = ALL:
##  sex response      SE df lower.CL upper.CL
##  F     0.0275 0.00502 14   0.0186   0.0407
##  M     0.0330 0.00603 14   0.0223   0.0488
##
## diet = COP:
##  sex response      SE df lower.CL upper.CL
##  F     0.0264 0.00482 14   0.0178   0.0390
##  M     0.0506 0.00924 14   0.0342   0.0749
##
## diet = SAK:
##  sex response      SE df lower.CL upper.CL
##  F     0.0242 0.00441 14   0.0163   0.0358
##  M     0.0388 0.00708 14   0.0262   0.0574
##
## diet = SKR:
##  sex response      SE df lower.CL upper.CL
##  F     0.0240 0.00439 14   0.0163   0.0356
##  M     0.0496 0.00905 14   0.0335   0.0734
##
## diet = wild:
##  sex response      SE df lower.CL upper.CL
##  F     0.0271 0.00343 20   0.0208   0.0353
##  M     0.0131 0.00165 20   0.0100   0.0170
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
## Intervals are back-transformed from the log scale
##
## $contrasts
## diet = BLW:
##  contrast ratio    SE df t.ratio p.value
##  F / M    0.480 0.124 14 -2.840  0.0131
##
## diet = ALL:
##  contrast ratio    SE df t.ratio p.value
##  F / M    0.832 0.215 14 -0.712  0.4884
##
## diet = COP:
##  contrast ratio    SE df t.ratio p.value
##  F / M    0.521 0.135 14 -2.525  0.0243
##
## diet = SAK:
##  contrast ratio    SE df t.ratio p.value
##  F / M    0.623 0.161 14 -1.833  0.0881
##
## diet = SKR:
##  contrast ratio    SE df t.ratio p.value
##  F / M    0.485 0.125 14 -2.807  0.0140
##
## diet = wild:
##  contrast ratio    SE df t.ratio p.value
##  F / M    2.077 0.214 74  7.094  <.0001
##
## Degrees-of-freedom method: kenward-roger
## Tests are performed on the log scale
```

SUPPORTING INFORMATION S12



SUPPORTING INFORMATION S12A: Visual presentation of age dependent amount of food consumed in relation to body mass. Note separate y-axis for bloodworm (on right). Observations were conducted on young adults at 30 – 91 dph. COP = Coppens Orange, ALR = Aller Infa, SAK = SAK MIX, SKR = Skretting Vitalis, BLW = bloodworm.

SUPPORTING INFORMATION S12B: Statistical results of the age dependent relative amount (% of bm) of food consumed. Linear permutation test.

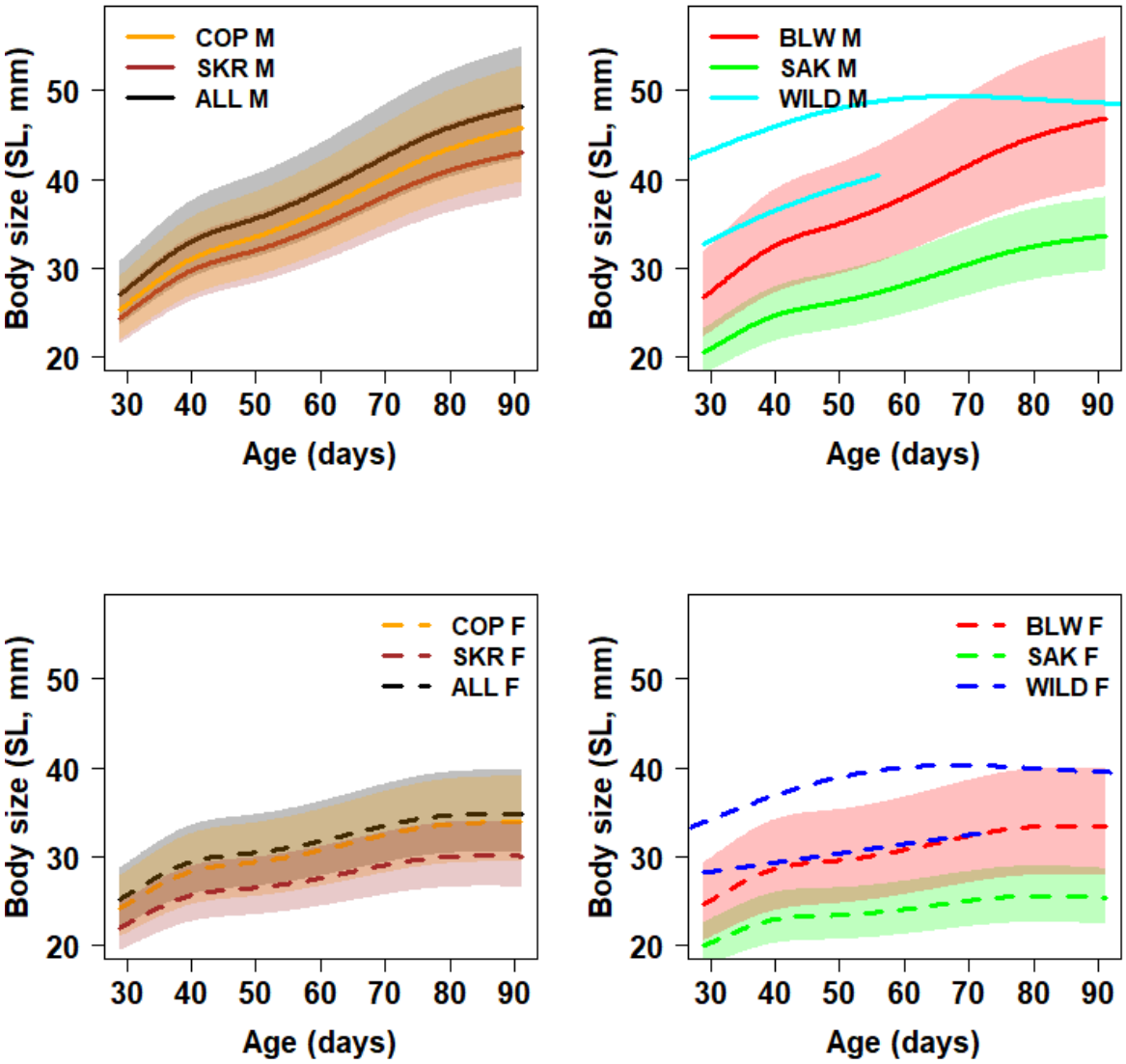
```
## Analysis of Variance Table
##
## Response: feed_bw
##           Df R Sum Sq R Mean Sq F value    Pr(>F)
## fage       1 0.001579 0.0015786  2.9758   0.09737 .
## diet       4 0.111264 0.0278159 52.4359 1.616e-11 ***
## Residuals 24 0.012731 0.0005305
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

SUPPORTING INFORMATION S13: Statistical results of age dependent body size/growth trajectory (Gaussian Generalized Additive Model).

```
##
## Family: gaussian
## Link function: identity
##
## Formula:
## log(sl) ~ s(age, k = 6) + s(age, by = diet, k = 6) + s(age, by = sex,
##     k = 6) + diet * sex + s(tank, bs = "re")
##
## Parametric Terms:
##          df       F  p-value
## diet      4   6.468 3.85e-05
## sex       1 293.453  < 2e-16
## diet:sex  4   7.346 7.81e-06
##
## Approximate significance of smooth terms:
##                   edf  Ref.df       F  p-value
## s(age)          4.4904  4.5833 568.743  < 2e-16
## s(age):dietALL  0.8235  0.8235  60.438  < 2e-16
## s(age):dietBLW  0.8235  0.8235  37.806  < 2e-16
## s(age):dietCOP  0.8235  0.8235  78.068  < 2e-16
## s(age):dietSAK  0.9816  1.1217   0.626    0.503
## s(age):dietSKR  0.8235  0.8235  47.722  < 2e-16
## s(age):sexF     0.6471  0.6471  31.271 7.73e-06
## s(age):sexM     0.6471  0.6471 812.350  < 2e-16
## s(tank)        18.2611 19.0000  22.588  < 2e-16
```

```
## $emmeans
## sex = F:
##  diet response   SE   df lower.CL upper.CL
##  ALL      31.1 1.58 1031     28.2     34.4
##  BLW      30.2 2.39 1031     25.8     35.3
##  COP      30.1 1.70 1031     26.9     33.6
##  SAK      23.8 1.07 1031     21.7     26.0
##  SKR      27.1 1.18 1031     24.9     29.5
##
## sex = M:
##  diet response   SE   df lower.CL upper.CL
##  ALL      37.3 1.87 1031     33.8     41.1
##  BLW      36.6 2.90 1031     31.4     42.8
##  COP      35.2 1.99 1031     31.5     39.3
##  SAK      27.3 1.22 1031     25.0     29.8
##  SKR      33.5 1.46 1031     30.8     36.5
##
## Results are averaged over the levels of: tank
## Confidence level used: 0.95
## Intervals are back-transformed from the log scale
##
## $contrasts
## sex = F:
##  contrast  ratio     SE   df t.ratio p.value
##  ALL / BLW 1.032 0.1160 1031  0.277  0.9987
##  ALL / COP 1.035 0.0887 1031  0.399  0.9947
##  ALL / SAK 1.311 0.0941 1031  3.771  0.0016
##  ALL / SKR 1.150 0.0561 1031  2.863  0.0347
##  BLW / COP 1.003 0.1156 1031  0.026  1.0000
##  BLW / SAK 1.271 0.1400 1031  2.174  0.1904
##  BLW / SKR 1.115 0.1221 1031  0.992  0.8591
##  COP / SAK 1.267 0.0796 1031  3.765  0.0016
##  COP / SKR 1.111 0.0870 1031  1.349  0.6603
##  SAK / SKR 0.877 0.0535 1031 -2.147  0.2010
##
## sex = M:
##  contrast  ratio     SE   df t.ratio p.value
##  ALL / BLW 1.018 0.1143 1031  0.159  0.9999
##  ALL / COP 1.060 0.0906 1031  0.684  0.9599
##  ALL / SAK 1.367 0.0965 1031  4.431  0.0001
##  ALL / SKR 1.113 0.0531 1031  2.246  0.1638
##  BLW / COP 1.041 0.1202 1031  0.352  0.9967
##  BLW / SAK 1.343 0.1476 1031  2.683  0.0572
##  BLW / SKR 1.093 0.1198 1031  0.815  0.9261
##  COP / SAK 1.290 0.0803 1031  4.082  0.0005
##  COP / SKR 1.050 0.0823 1031  0.620  0.9718
##  SAK / SKR 0.814 0.0490 1031 -3.419  0.0059
##
## Results are averaged over the levels of: tank
## P value adjustment: tukey method for comparing a family of 5 estimates
## Tests are performed on the log scale
```

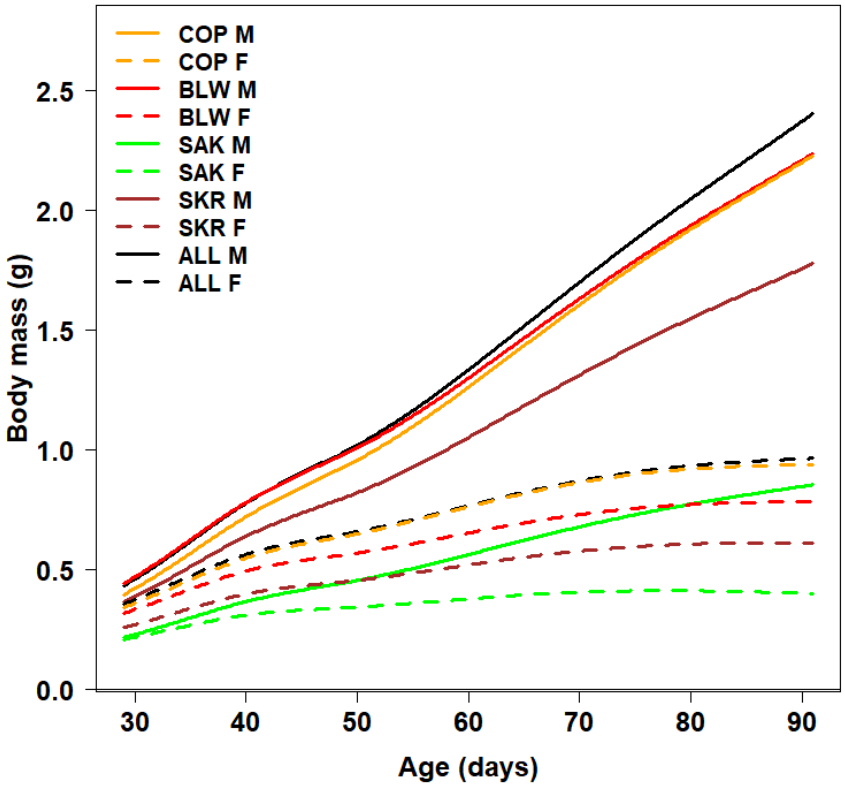
SUPPORTING INFORMATION S14: Growth curves of *Nothobranchius furzeri* (age dependent SL)



SUPPORTING INFORMATION S14: Age-dependent body size (standard length) of adults (age > 29 days). Curves produced from Gaussian Generalized Additive Models. Curves for wild fish from Vrtílek et al., (2019) are presented only for visual comparison and were not included in statistical comparison. Plots are split into four figures for better clarity of 95% Confidence intervals.

SUPPORTING INFORMATION S15: Commentary on results for age dependent body mass

Growth in body mass was diet-dependent in a sex-specific manner (Gaussian GAM, diet:sex interaction: $F_{4,1040} = 17.99$, $p < 0.001$, Figure below). In males, the Aller (multiple comparison ratio (se), 2.31 (0.47), $t_{1040} = 4.09$, $p = 0.002$), Coppens (2.18 (0.39), $t_{1040} = 4.35$, $p = 0.001$) and Skretting (0.54 (0.09), $t_{1040} = 3.52$, $p = 0.016$) groups had significantly better growth than the SAK group. In females, only the Aller (1.98 (0.41), $t_{1040} = 3.28$, $p = 0.035$) and Coppens (1.96 (0.35), $t_{1040} = 3.73$, $p = 0.008$) groups outperformed SAK. Other comparisons were not significant (males: 0.74 – 1.70 (0.13-0.59), $t_{1040} = 0.04$-2.73, $p = 0.162$-1.000; females: 1.02-2.27 (0.17-0.78), $t_{1040} = 0.06$-2.34, $p = 0.342$-1.000).



SUPPORTING INFORMATION S15: Age dependent body mass. Curves are produced by Gaussian GAM. Cop – Coppens Orange, BLW – bloodworms, SAK – SAK MIX, SKR – Skretting Vitalis, ALL – Aller Infa; M – male, F – female.

SUPPORTING INFORMATION S16: Comments on results for juvenile growth

Juvenile growth in body mass (i.e. body size at 29dph) was better in the Aller-fed group than in the Coppens (pairwise ratio (se), 1.539 (0.164), $t_{14.8} = 4.04$, $p = 0.008$) and SAK-fed groups (1.442 (0.152), $t_{14.9} = 3.30$, $p = 0.034$) and did not differ from bloodworm (1.057 (0.113), $t_{14.8} = 0.52$, $p = 0.985$) or Skretting (1.079 (0.115), $t_{14.8} = 0.716$, $p = 0.950$) groups.

SUPPORTING INFORMATION S17: Statistical results of the Thermal-Unit Growth Coefficient (Gaussian Linear Model).

```
##
## Call:
## lm(formula = log(TGC) ~ age + diet + sex, data = totgc)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.11607 -0.25887  0.03195  0.32533  0.91861
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  2.450077   0.225675  10.857 4.39e-15 ***
## age         -0.027552   0.002884  -9.553 4.07e-13 ***
## dietBLW     -0.083991   0.195551  -0.430  0.66929
## dietCOP     -0.304525   0.195551  -1.557  0.12536
## dietSAK     -0.556726   0.195551  -2.847  0.00627 **
## dietSKR     -0.238307   0.195551  -1.219  0.22837
## sexM         0.693893   0.123677   5.611 7.49e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.479 on 53 degrees of freedom
## Multiple R-squared:  0.7142, Adjusted R-squared:  0.6819
## F-statistic: 22.08 on 6 and 53 DF,  p-value: 7.891e-13
```

```
## $emmeans
##  diet response    SE df lower.CL upper.CL
##  ALL      3.32 0.459 53     2.51     4.38
##  BLW      3.05 0.422 53     2.31     4.02
##  COP      2.45 0.338 53     1.85     3.23
##  SAK      1.90 0.263 53     1.44     2.51
##  SKR      2.61 0.361 53     1.98     3.45
##
## Results are averaged over the levels of: sex
## Confidence level used: 0.95
## Intervals are back-transformed from the log scale
##
## $contrasts
##  contrast  ratio    SE df t.ratio p.value
##  ALL / BLW 1.088 0.213 53  0.430  0.9927
##  ALL / COP 1.356 0.265 53  1.557  0.5308
##  ALL / SAK 1.745 0.341 53  2.847  0.0472
##  ALL / SKR 1.269 0.248 53  1.219  0.7406
##  BLW / COP 1.247 0.244 53  1.128  0.7912
##  BLW / SAK 1.604 0.314 53  2.417  0.1265
##  BLW / SKR 1.167 0.228 53  0.789  0.9327
##  COP / SAK 1.287 0.252 53  1.290  0.6986
##  COP / SKR 0.936 0.183 53 -0.339  0.9971
##  SAK / SKR 0.727 0.142 53 -1.628  0.4864
##
## Results are averaged over the levels of: sex
## P value adjustment: tukey method for comparing a family of 5 estimates
## Tests are performed on the log scale
```

SUPPORTING INFORMATION S18: Fish survival. (Cox mixed effects model.)

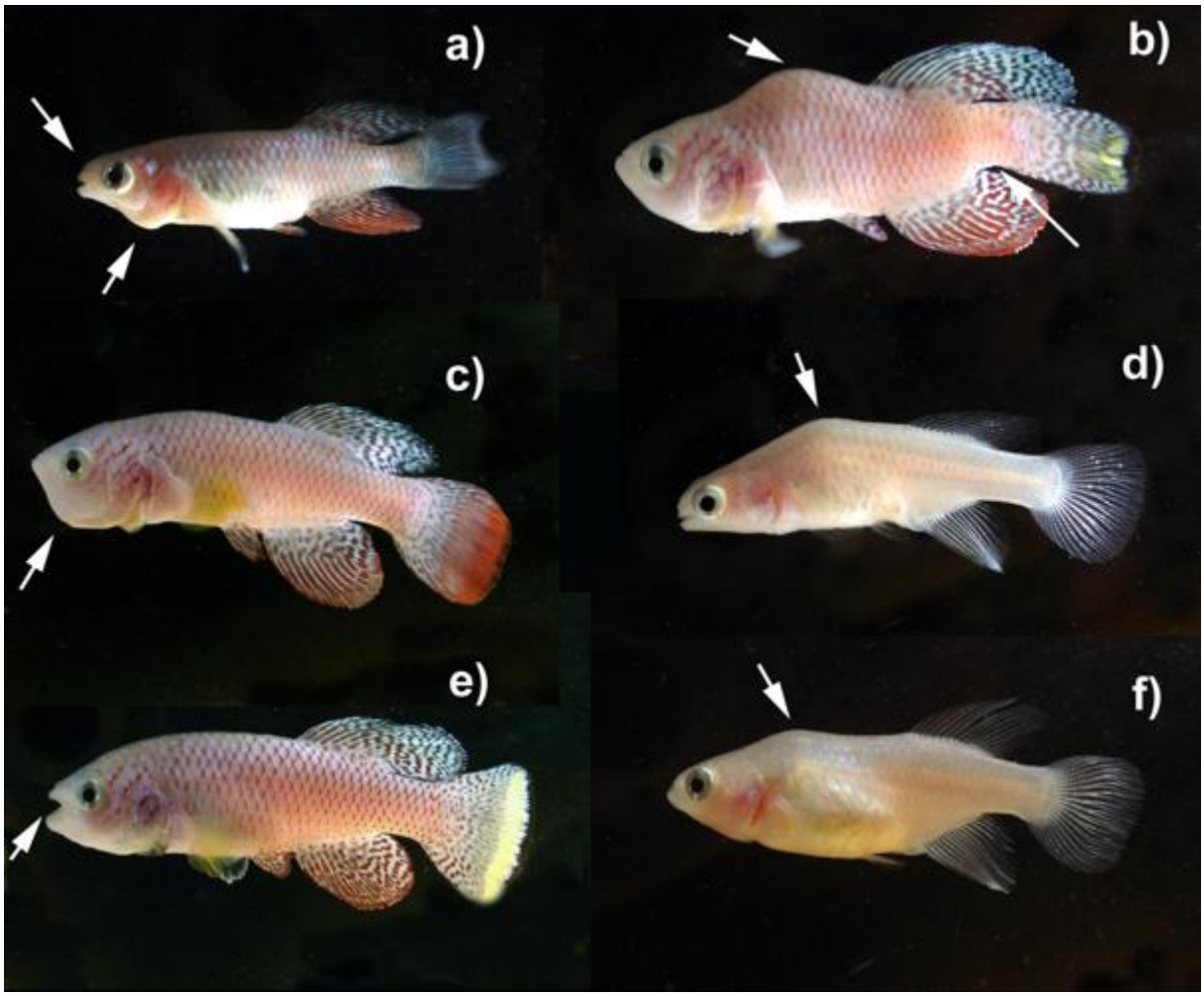
```
summary(mps)
```

```
## Cox mixed-effects model fit by maximum likelihood
##   Data: prez
##   events, n = 53, 240
##   Iterations= 5 22
##                     NULL Integrated    Fitted
## Log-likelihood -284.2607  -282.6057 -282.6022
##
##                   Chisq df       p   AIC    BIC
## Integrated loglik  3.31  6 0.76906 -8.69 -20.51
##  Penalized loglik  3.32  5 0.65170 -6.69 -16.55
##
## Model:  Surv(age, death, type = "right") ~ diet + sex + (1 | tank)
## Fixed coefficients
##                      coef exp(coef)  se(coef)    z    p
## dietALLER     0.50311709  1.653869 0.4338121 1.16 0.25
## dietCOPPENS   0.45462227  1.575578 0.4411466 1.03 0.30
## dietSAK       0.11471983  1.121559 0.4609359 0.25 0.80
## dietSKRETTING 0.09717562  1.102054 0.4714871 0.21 0.84
## sexM          0.26800684  1.307356 0.2763970 0.97 0.33
##
## Random effects
##  Group Variable  Std Dev      Variance
##  tank  Intercept 9.059939e-03 8.208249e-05
```

```
Anova(mps)
```

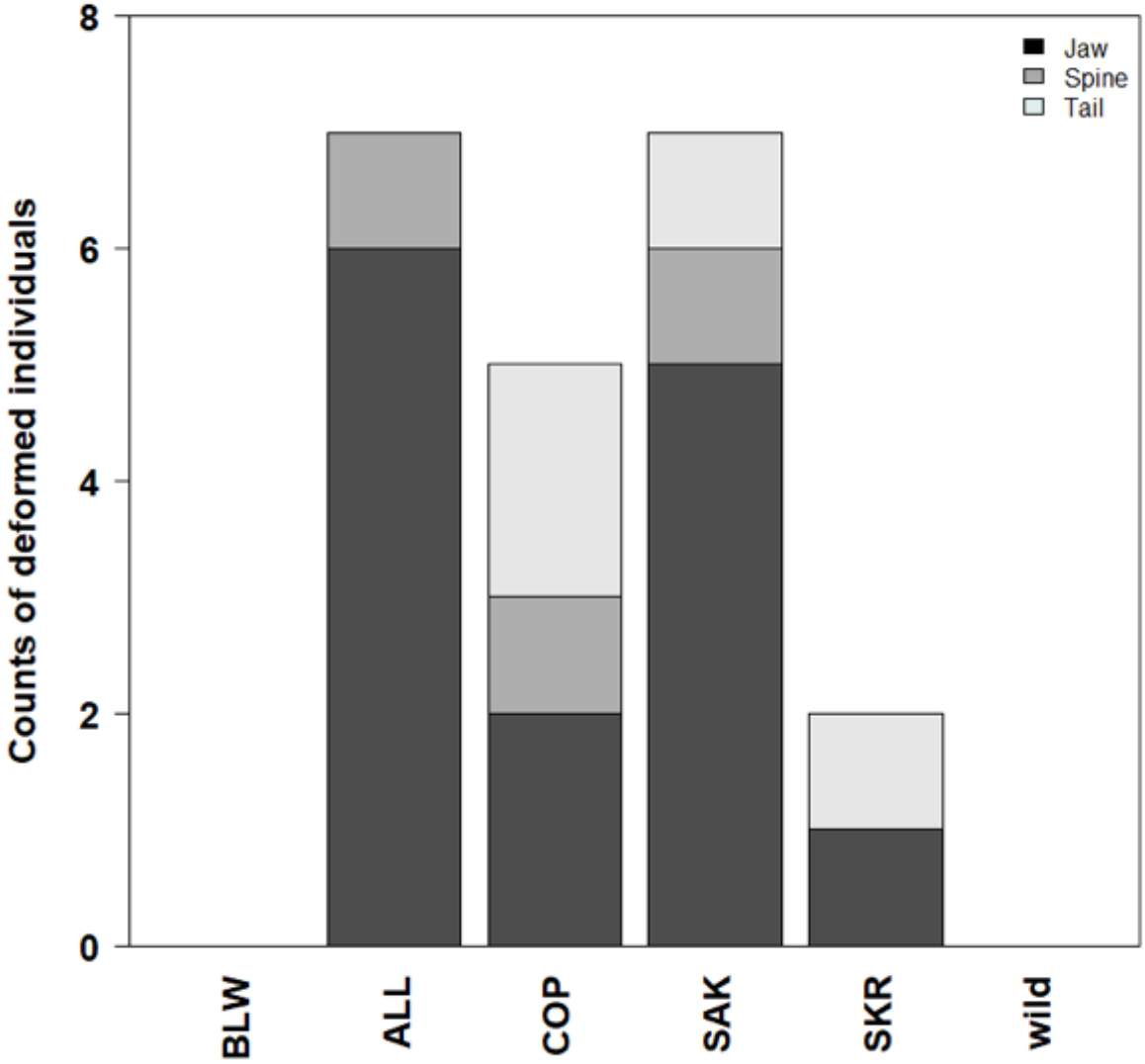
```
## Analysis of Deviance Table (Type II tests)
##
## Response: Surv(age, death, type = "right")
##      Df  Chisq Pr(>Chisq)
## diet  4 2.2749     0.6853
## sex   1 0.9402     0.3322
```

SUPPORTING INFORMATION S19



SUPPORTING INFORMATION S19: Bone deformities observed in the experiment. a) Jaw and operculum deformity of male fed SAK MIX diet. This deformity complicated food acceptance. b) Spine and tail deformity of male fed Coppens Orange. This deformity complicates swimming because the spine is deformed both laterally and vertically. The condition does not influence feeding or spawning behaviour. c) Opercular deformity of male fed Aller Infa. This deformity limits the mouth gape and thus causes difficulties during feeding. d) Spine deformity in female fed Aller Infa. This deformity has no apparent effect on fish because spine is deformed only vertically. e) Jaw deformity in male fed Skretting Vitalis preventing jaw closure; fish have permanently opened mouths but without apparent consequences for food acceptance. This deformity is sometimes also seen in fish fed bloodworm (Žák pers. obs), f) Spine deformity in female fed Aller Infa. This deformity led to slower swimming but had no apparent consequences for feeding or spawning.

SUPPORTING INFORMATION S20



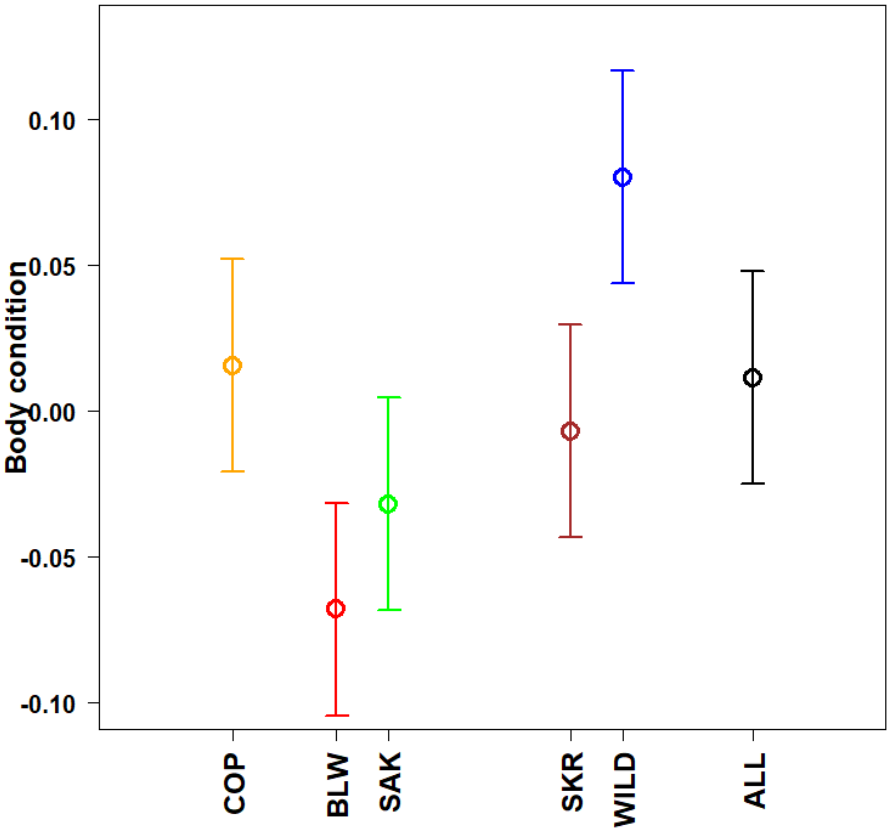
SUPPORTING INFORMATION S20: Counts and proportions of all recognized diet-associated bone deformities. Jaw deformities were the most frequently observed deformity and spine and tail deformities were relatively rare. Spine and tail deformity are distinguished as: spine deformity – deformity of vertebrae column from head to the beginning of the caudal peduncle (SUPPORTING INFORMATION S17 b,d,f); tail deformity – deformity of spine or hypuralia at the caudal peduncle (see SUPPORTING INFORMATION S17b). Cop – Coppens Orange, BLW – bloodworms, SAK – SAK MIX, SKR – Skretting Vitalis, ALR – Aller Infa; M – male, F – female.

SUPPORTING INFORMATION S21: Body condition statistical results. (Gaussian Linear Model)

```
##
## Call:
## lm(formula = resid.sl ~ diet + sex, data = levrt)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.22501 -0.05192 -0.00361  0.05578  0.41554
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.0299379  0.0170949   1.751  0.08164 .
## dietBLW     -0.0674058  0.0227098  -2.968  0.00341 **
## dietCOP     -0.0374565  0.0228773  -1.637  0.10336
## dietSAK     -0.0641102  0.0226942  -2.825  0.00527 **
## dietSKR     -0.0338042  0.0227098  -1.489  0.13840
## dietwild     0.0421770  0.0245270   1.720  0.08726 .
## sexM        -0.0004592  0.0134684  -0.034  0.97284
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.09078 on 176 degrees of freedom
## Multiple R-squared:  0.141,  Adjusted R-squared:  0.1117
## F-statistic: 4.814 on 6 and 176 DF,  p-value: 0.0001422
```

```
## $emmeans
##  diet    emmean     SE  df lower.CL upper.CL
##  ALL   0.02971 0.0161 176  -0.0020  0.06142
##  BLW  -0.03770 0.0160 176  -0.0694 -0.00603
##  COP  -0.00775 0.0163 176  -0.0400  0.02445
##  SAK  -0.03440 0.0161 176  -0.0661 -0.00269
##  SKR  -0.00410 0.0160 176  -0.0358  0.02757
##  wild  0.07189 0.0185 176   0.0353  0.10845
##
## Results are averaged over the levels of: sex
## Confidence level used: 0.95
##
## $contrasts
##  contrast   estimate     SE  df t.ratio p.value
##  ALL - BLW   0.06741 0.0227 176  2.968  0.0393
##  ALL - COP   0.03746 0.0229 176  1.637  0.5751
##  ALL - SAK   0.06411 0.0227 176  2.825  0.0582
##  ALL - SKR   0.03380 0.0227 176  1.489  0.6721
##  ALL - wild -0.04218 0.0245 176 -1.720  0.5207
##  BLW - COP  -0.02995 0.0229 176 -1.309  0.7800
##  BLW - SAK  -0.00330 0.0227 176 -0.145  1.0000
##  BLW - SKR  -0.03360 0.0227 176 -1.481  0.6771
##  BLW - wild -0.10958 0.0245 176 -4.470  0.0002
##  COP - SAK   0.02665 0.0229 176  1.165  0.8528
##  COP - SKR  -0.00365 0.0229 176 -0.160  1.0000
##  COP - wild -0.07963 0.0247 176 -3.225  0.0185
##  SAK - SKR  -0.03031 0.0227 176 -1.334  0.7654
##  SAK - wild -0.10629 0.0245 176 -4.333  0.0004
##  SKR - wild -0.07598 0.0245 176 -3.100  0.0270
##
## Results are averaged over the levels of: sex
## P value adjustment: tukey method for comparing a family of 6 estimates
```

SUPPORTING INFORMATION S22



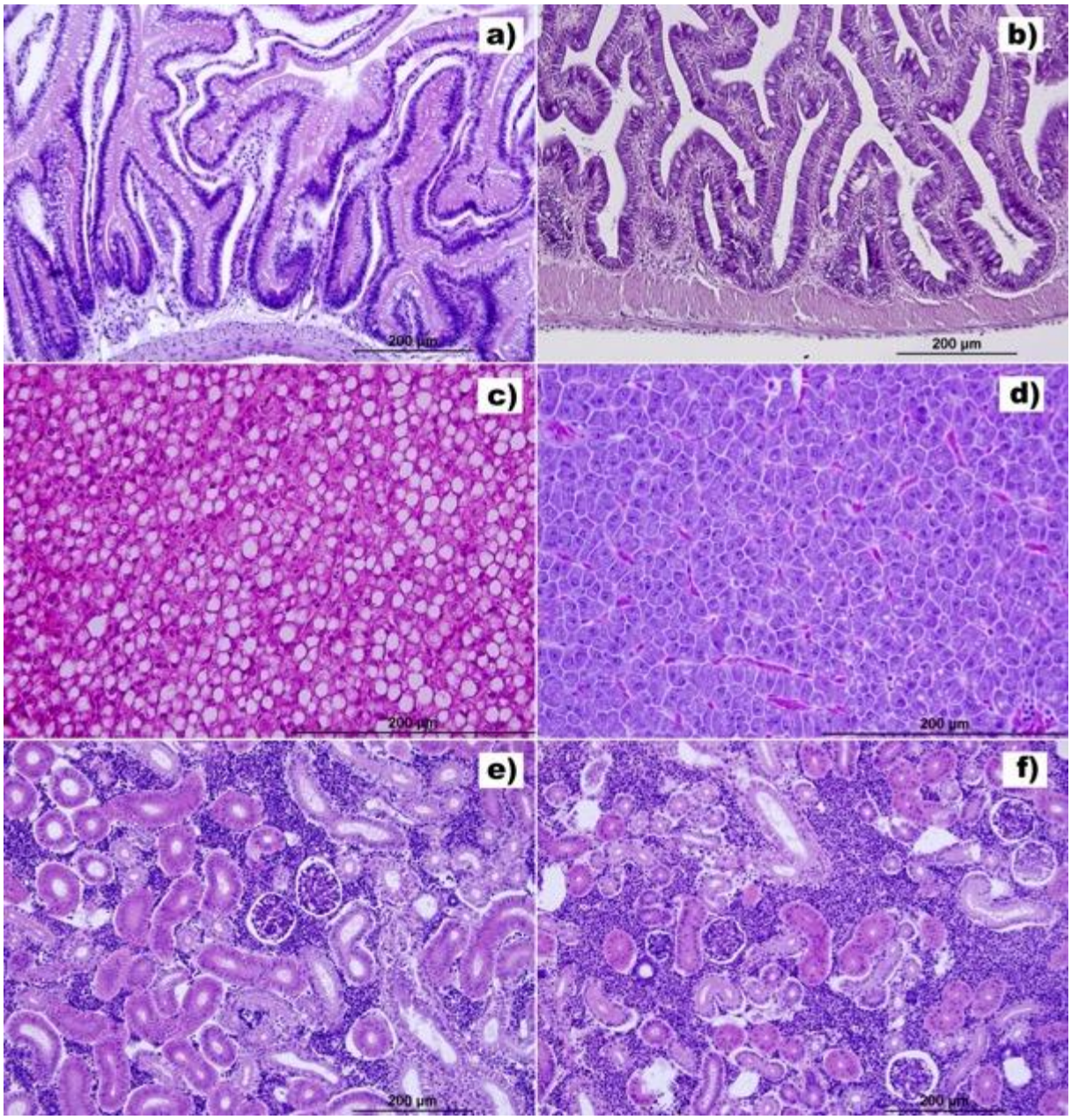
SUPPORTING INFORMATION S22: The relationship between diet and body condition with eviscerated body mass used as a measure of weight. Error bars are 95% Confidence intervals. Analysed by LMM. Cop – Coppens Orange, BLW – bloodworms, SAK – SAK MIX, SKR – Skretting Vitalis, ALL – Aller Infa. X axis is sorted in accordance with protein concentration in each food.

SUPPORTING INFORMATION S23: Statistical results of hepatocellular vacuolation. Ordinal GAM.

```
##
## Family: Ordered Categorical(-1,2,5.31)
## Link function: identity
##
## Formula:
## vac_score2 ~ diet + sex + s(tank, bs = "re")
##
## Parametric coefficients:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept)   2.2827     1.1401   2.002 0.045266 *
## dietALL      -3.1758     1.3839  -2.295 0.021743 *
## dietCOP       1.4572     1.5621   0.933 0.350879
## dietSAK      -3.0336     1.3981  -2.170 0.030029 *
## dietSKR      -2.1637     1.3443  -1.610 0.107494
## dietWILD     -4.8783     1.4195  -3.437 0.000589 ***
## sexM          5.7035     0.7058   8.081 6.45e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Approximate significance of smooth terms:
##           edf Ref.df Chi.sq p-value
## s(tank) 1.827     18  2.171   0.266
##
## Deviance explained = 61.9%
## -REML = 30.368  Scale est. = 1         n = 46
```

```
## $emmeans
##  diet emmean    SE   df lower.CL upper.CL
##  BLW   5.134 1.097 37.2    2.911     7.36
##  ALL   1.959 0.737 37.2    0.466     3.45
##  COP   6.592 1.083 37.2    4.397     8.79
##  SAK   2.101 0.772 37.2    0.536     3.67
##  SKR   2.971 0.678 37.2    1.597     4.34
##  WILD  0.256 0.791 37.2   -1.346     1.86
##
## Results are averaged over the levels of: sex, tank
## Confidence level used: 0.95
##
## $contrasts
##  contrast   estimate   SE   df t.ratio p.value
##  BLW - ALL     3.176 1.33 37.2  2.393  0.1849
##  BLW - COP    -1.457 1.51 37.2 -0.964  0.9264
##  BLW - SAK     3.034 1.34 37.2  2.261  0.2358
##  BLW - SKR     2.164 1.29 37.2  1.683  0.5512
##  BLW - WILD    4.878 1.36 37.2  3.576  0.0118
##  ALL - COP    -4.633 1.32 37.2 -3.507  0.0141
##  ALL - SAK    -0.142 1.07 37.2 -0.133  1.0000
##  ALL - SKR    -1.012 1.00 37.2 -1.009  0.9119
##  ALL - WILD    1.702 1.08 37.2  1.582  0.6151
##  COP - SAK     4.491 1.33 37.2  3.376  0.0199
##  COP - SKR     3.621 1.27 37.2  2.853  0.0708
##  COP - WILD    6.336 1.36 37.2  4.643  0.0006
##  SAK - SKR    -0.870 1.03 37.2 -0.846  0.9565
##  SAK - WILD    1.845 1.11 37.2  1.669  0.5601
##  SKR - WILD    2.715 1.05 37.2  2.597  0.1233
##
## Results are averaged over the levels of: sex, tank
## P value adjustment: tukey method for comparing a family of 6 estimates
```

SUPPORTING INFORMATION S24



SUPPORTING INFORMATION S24: Histology of organs in experimental (a,c,e,f) and wild (b,d) *Nothobranchius furzeri*. Intestine (a,b). Liver (c,d), Kidney (e,f). a) Intestine of experimental male fed SAK MIX diet. b) Intestine of wild fish. c). Hepatocellular vacuolation in liver of male fed Skretting Vitalis. d) Liver parenchyma of wild female from free of hepatocellular vacuoles. e) Kidney of male fed Aller Infa. f) Kidney of male fed bloodworm.

SUPPORTING INFORMATION S25A: Statistical results of diet dependent hepato-somatic index. Linear mixed effect model.

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: hsi ~ diet * sex + (1 | tank)
##    Data: dis2
##
## REML criterion at convergence: 303.4
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.4095 -0.5672 -0.1434  0.6319  2.2568
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  tank     (Intercept) 0.03379  0.1838
##  Residual             0.44990  0.6707
## Number of obs: 143, groups:  tank, 24
##
## Fixed effects:
##               Estimate Std. Error t value
## (Intercept)    4.00590    0.20175  19.856
## dietBLW        0.10583    0.29435   0.360
## dietCOP       -1.04731    0.28532  -3.671
## dietSAK       -2.19269    0.28532  -7.685
## dietSKR       -0.43980    0.29435  -1.494
## dietwild       0.06582    0.29435   0.224
## sexM          -2.38974    0.27835  -8.585
## dietBLW:sexM   1.55228    0.39046   3.975
## dietCOP:sexM   1.58100    0.39407   4.012
## dietSAK:sexM   1.99630    0.39407   5.066
## dietSKR:sexM   0.77350    0.39499   1.958
## dietwild:sexM  0.05119    0.39046   0.131
##
## Correlation of Fixed Effects:
##             (Intr) ditBLW ditCOP ditSAK ditSKR ditwld sexM   dBLW:M dCOP:M
## dietBLW     -0.685
## dietCOP     -0.707  0.485
## dietSAK     -0.707  0.485  0.500
## dietSKR     -0.685  0.470  0.485  0.485
## dietwild    -0.685  0.470  0.485  0.485  0.470
## sexM        -0.574  0.394  0.406  0.406  0.394  0.394
## dietBLW:sxM  0.409 -0.607 -0.290 -0.290 -0.281 -0.281 -0.713
## dietCOP:sxM  0.406 -0.278 -0.575 -0.287 -0.278 -0.278 -0.706  0.504
## dietSAK:sxM  0.406 -0.278 -0.287 -0.575 -0.278 -0.278 -0.706  0.504  0.499
## dietSKR:sxM  0.405 -0.277 -0.286 -0.286 -0.600 -0.277 -0.705  0.502  0.498
## dietwld:sxM  0.409 -0.281 -0.290 -0.290 -0.281 -0.607 -0.713  0.508  0.504
##             dSAK:M dSKR:M
## dietBLW
## dietCOP
## dietSAK
## dietSKR
## dietwild
## sexM
## dietBLW:sxM
## dietCOP:sxM
## dietSAK:sxM
## dietSKR:sxM  0.498
## dietwld:sxM  0.504  0.502
```

SUPPORTING INFORMATION S25B: Pairwise comparisons of the statistical results for diet dependent hepato-somatic index.

```
## $emmeans
## sex = F:
##  diet emmean    SE   df lower.CL upper.CL
##  ALL    4.01 0.202 37.6    3.597     4.41
##  BLW    4.11 0.214 47.1    3.681     4.54
##  COP    2.96 0.202 37.5    2.550     3.37
##  SAK    1.81 0.202 37.5    1.404     2.22
##  SKR    3.57 0.214 47.1    3.135     4.00
##  wild   4.07 0.214 47.1    3.641     4.50
##
## sex = M:
##  diet emmean    SE   df lower.CL upper.CL
##  ALL    1.62 0.232 58.5    1.152     2.08
##  BLW    3.27 0.214 47.1    2.843     3.71
##  COP    2.15 0.232 58.5    1.686     2.61
##  SAK    1.42 0.232 58.5    0.956     1.88
##  SKR    1.95 0.223 52.1    1.503     2.40
##  wild   1.73 0.214 47.1    1.302     2.16
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
## sex = F:
##  contrast   estimate    SE   df t.ratio p.value
##  ALL - BLW   -0.1058 0.294 42.3 -0.359  0.9992
##  ALL - COP    1.0473 0.286 37.6  3.668  0.0091
##  ALL - SAK    2.1927 0.286 37.6  7.679  <.0001
##  ALL - SKR    0.4398 0.294 42.3  1.494  0.6701
##  ALL - wild  -0.0658 0.294 42.3 -0.224  0.9999
##  BLW - COP    1.1531 0.294 42.3  3.916  0.0041
##  BLW - SAK    2.2985 0.294 42.3  7.806  <.0001
##  BLW - SKR    0.5456 0.303 47.1  1.800  0.4755
##  BLW - wild   0.0400 0.303 47.1  0.132  1.0000
##  COP - SAK    1.1454 0.286 37.5  4.011  0.0035
##  COP - SKR   -0.6075 0.294 42.3 -2.063  0.3257
##  COP - wild  -1.1131 0.294 42.3 -3.780  0.0061
##  SAK - SKR   -1.7529 0.294 42.3 -5.953  <.0001
##  SAK - wild  -2.2585 0.294 42.3 -7.670  <.0001
##  SKR - wild  -0.5056 0.303 47.1 -1.668  0.5590
```

```
##
## sex = M:
##  contrast   estimate    SE   df t.ratio p.value
##  ALL - BLW   -1.6581 0.316 52.9 -5.250  <.0001
##  ALL - COP   -0.5337 0.328 58.5 -1.627  0.5845
##  ALL - SAK    0.1964 0.328 58.5  0.599  0.9907
##  ALL - SKR   -0.3337 0.322 55.3 -1.038  0.9030
##  ALL - wild  -0.1170 0.316 52.9 -0.370  0.9990
##  BLW - COP    1.1244 0.316 52.9  3.560  0.0098
##  BLW - SAK    1.8545 0.316 52.9  5.872  <.0001
##  BLW - SKR    1.3244 0.309 49.6  4.285  0.0011
##  BLW - wild   1.5411 0.303 47.1  5.084  0.0001
##  COP - SAK    0.7301 0.328 58.5  2.226  0.2420
##  COP - SKR    0.2000 0.322 55.3  0.622  0.9889
##  COP - wild   0.4167 0.316 52.9  1.319  0.7731
##  SAK - SKR   -0.5301 0.322 55.3 -1.649  0.5707
##  SAK - wild  -0.3134 0.316 52.9 -0.992  0.9184
##  SKR - wild   0.2167 0.309 49.6  0.701  0.9810
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 6 estimates
```

SUPPORTING INFORMATION S26. Statistical results of visceral fat score (Ordinal GAM)

```
##
## Family: Ordered Categorical(-1,2.77,5.06)
## Link function: identity
##
## Formula:
## vf2 ~ diet + sex + s(tank, bs = "re")
##
## Parametric coefficients:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept)   1.2943     0.5816   2.225   0.0260 *
## dietBLW       0.9392     0.7695   1.221   0.2223
## dietCOP      -1.3456     0.7869  -1.710   0.0873 .
## dietSAK      -3.6112     0.8374  -4.312 1.62e-05 ***
## dietSKR      -0.5462     0.8015  -0.682   0.4955
## sexM          2.4399     0.3506   6.959 3.43e-12 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Approximate significance of smooth terms:
##           edf Ref.df Chi.sq p-value
## s(tank) 7.637     15  14.49  0.0197 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Deviance explained = 38.5%
## -REML = 129.85  Scale est. = 1         n = 141
```

```
## $emmeans
##  diet emmean    SE  df lower.CL upper.CL
##  ALL    2.51 0.391 127    1.741    3.287
##  BLW    3.45 0.338 127    2.785    4.122
##  COP    1.17 0.378 127    0.421    1.916
##  SAK   -1.10 0.474 127   -2.035   -0.159
##  SKR    1.97 0.405 127    1.166    2.770
##
## Results are averaged over the levels of: sex, tank
## Confidence level used: 0.95
##
## $contrasts
##  contrast  estimate    SE  df t.ratio p.value
##  ALL - BLW   -0.939 0.517 127 -1.815  0.3693
##  ALL - COP    1.346 0.543 127  2.479  0.1020
##  ALL - SAK    3.611 0.614 127  5.883  <.0001
##  ALL - SKR    0.546 0.564 127  0.969  0.8687
##  BLW - COP    2.285 0.507 127  4.504  0.0001
##  BLW - SAK    4.550 0.582 127  7.813  <.0001
##  BLW - SKR    1.485 0.527 127  2.820  0.0435
##  COP - SAK    2.266 0.606 127  3.740  0.0025
##  COP - SKR   -0.799 0.555 127 -1.441  0.6022
##  SAK - SKR   -3.065 0.624 127 -4.912  <.0001
##
## Results are averaged over the levels of: sex, tank
## P value adjustment: tukey method for comparing a family of 5 estimates
```

SUPPORTING INFORMATION S27: Statistical results for gut length comparison. Linear mixed effect model.

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: rGL ~ diet + sex + gf + (1 | tank)
##    Data: gutlen
##
## REML criterion at convergence: -322.7
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.13320 -0.60770 -0.05997  0.60079  3.14434
##
## Random effects:
##  Groups   Name        Variance  Std.Dev.
##  tank     (Intercept) 0.0008679 0.02946
##  Residual             0.0038313 0.06190
## Number of obs: 144, groups:  tank, 24
##
## Fixed effects:
##               Estimate Std. Error t value
## (Intercept)  0.4949237  0.0189829  26.072
## dietBLW      0.0371571  0.0267483   1.389
## dietCOP      0.0332299  0.0265485   1.252
## dietSAK      0.0359924  0.0256687   1.402
## dietSKR     -0.0106537  0.0252107  -0.423
## dietWILD     0.0684707  0.0392146   1.746
## sexM        -0.0391994  0.0105970  -3.699
## gf           0.0010341  0.0004681   2.209
##
## Correlation of Fixed Effects:
##          (Intr) ditBLW ditCOP ditSAK ditSKR dtWILD sexM
## dietBLW  -0.661
## dietCOP  -0.676  0.476
## dietSAK  -0.683  0.485  0.513
## dietSKR  -0.650  0.460  0.465  0.493
## dietWILD -0.427  0.325  0.322  0.331  0.314
## sexM     -0.246 -0.023  0.022 -0.004  0.004 -0.113
## gf       -0.033 -0.003  0.003 -0.001  0.001 -0.731  0.134
```

SUPPORTING INFORMATION S28: Statistical results for gonadosomatic index (LME).

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: gsi ~ diet + (1 | tank)
##    Data: dis3
##
## REML criterion at convergence: 505.9
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.6559 -0.4415 -0.1283  0.5074  4.1866
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  tank     (Intercept)  6.409   2.532
##  Residual             48.769   6.983
## Number of obs: 78, groups:  tank, 24
##
## Fixed effects:
##             Estimate Std. Error t value
## (Intercept)   17.580      2.380   7.385
## dietALL        5.136      3.283   1.565
## dietCOP       -3.614      3.283  -1.101
## dietSAK       -6.574      3.283  -2.003
## dietSKR       -2.100      3.366  -0.624
## dietwild       1.254      3.366   0.372
##
## Correlation of Fixed Effects:
##          (Intr) ditALL ditCOP ditSAK ditSKR
## dietALL  -0.725
## dietCOP  -0.725  0.526
## dietSAK  -0.725  0.526  0.526
## dietSKR  -0.707  0.513  0.513  0.513
## dietwild -0.707  0.513  0.513  0.513  0.500
```

```
## $emmeans
##  diet emmean   SE   df lower.CL upper.CL
##  BLW    17.6 2.38 20.1    12.61     22.5
##  ALL    22.7 2.26 16.0    17.92     27.5
##  COP    14.0 2.26 16.0     9.17     18.8
##  SAK    11.0 2.26 16.0     6.21     15.8
##  SKR    15.5 2.38 20.1    10.51     20.4
##  wild   18.8 2.38 20.1    13.87     23.8
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast   estimate   SE   df t.ratio p.value
##  BLW - ALL     -5.14 3.28 18.0 -1.563  0.6308
##  BLW - COP      3.61 3.28 18.0  1.100  0.8750
##  BLW - SAK      6.57 3.28 18.0  2.001  0.3792
##  BLW - SKR      2.10 3.37 20.1  0.624  0.9879
##  BLW - wild    -1.25 3.37 20.1 -0.372  0.9989
##  ALL - COP      8.75 3.20 16.0  2.733  0.1223
##  ALL - SAK     11.71 3.20 16.0  3.658  0.0216
##  ALL - SKR      7.24 3.28 18.0  2.203  0.2839
##  ALL - wild     3.88 3.28 18.0  1.182  0.8397
##  COP - SAK      2.96 3.20 16.0  0.925  0.9343
##  COP - SKR     -1.51 3.28 18.0 -0.461  0.9970
##  COP - wild    -4.87 3.28 18.0 -1.482  0.6794
##  SAK - SKR     -4.47 3.28 18.0 -1.362  0.7479
##  SAK - wild    -7.83 3.28 18.0 -2.383  0.2138
##  SKR - wild    -3.35 3.37 20.1 -0.996  0.9138
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 6 estimates
```

SUPPORTING INFORMATION S29: Statistical results for absolute fecundity. Negative binomial GLMM.

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(1.0917)  ( log )
## Formula: totN ~ diet + (1 | tankF)
##    Data: repr
##
##      AIC      BIC   logLik deviance df.resid
##    792.5    813.0   -388.3    776.5       88
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.0115 -0.6159 -0.1808  0.5964  2.1430
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  tankF  (Intercept) 0.01924  0.1387
## Number of obs: 96, groups:  tankF, 12
##
## Fixed effects:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept)  3.67101    0.26169  14.028  < 2e-16 ***
## dietALL     -0.04664    0.37026  -0.126   0.8998
## dietCOP     -0.90846    0.37408  -2.429   0.0152 *
## dietSAK     -1.51020    0.37756  -4.000 6.34e-05 ***
## dietSKR     -0.47275    0.38619  -1.224   0.2209
## dietwild    -1.08342    0.45098  -2.402   0.0163 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr) ditALL ditCOP ditSAK ditSKR
## dietALL  -0.707
## dietCOP  -0.699  0.495
## dietSAK  -0.693  0.490  0.485
## dietSKR  -0.676  0.482  0.488  0.471
## dietwild -0.576  0.416  0.435  0.405  0.534
```

```
## $emmeans
##  diet response    SE  df asymp.LCL asymp.UCL
##  BLW     39.29 10.28 Inf     23.53      65.6
##  ALL     37.50  9.82 Inf     22.44      62.7
##  COP     15.84  4.24 Inf      9.38      26.8
##  SAK      8.68  2.36 Inf      5.09      14.8
##  SKR     24.49  6.97 Inf     14.02      42.8
##  wild    13.30  4.90 Inf      6.46      27.4
##
## Confidence level used: 0.95
## Intervals are back-transformed from the log scale
##
## $contrasts
##  contrast   ratio    SE  df z.ratio p.value
##  BLW / ALL  1.048 0.388 Inf  0.126  1.0000
##  BLW / COP  2.480 0.928 Inf  2.429  0.1464
##  BLW / SAK  4.528 1.709 Inf  4.000  0.0009
##  BLW / SKR  1.604 0.620 Inf  1.224  0.8252
##  BLW / wild 2.955 1.333 Inf  2.402  0.1553
##  ALL / COP  2.367 0.886 Inf  2.304  0.1923
##  ALL / SAK  4.321 1.632 Inf  3.875  0.0015
##  ALL / SKR  1.531 0.590 Inf  1.106  0.8792
##  ALL / wild 2.820 1.266 Inf  2.309  0.1903
##  COP / SAK  1.825 0.696 Inf  1.578  0.6133
##  COP / SKR  0.647 0.249 Inf -1.132  0.8682
##  COP / wild 1.191 0.528 Inf  0.395  0.9988
##  SAK / SKR  0.354 0.139 Inf -2.641  0.0876
##  SAK / wild 0.653 0.298 Inf -0.936  0.9373
##  SKR / wild 1.842 0.751 Inf  1.497  0.6664
##
## P value adjustment: tukey method for comparing a family of 6 estimates
## Tests are performed on the log scale
```

SUPPORTING INFORMATION S30: Statistical results for fecundity corrected for female body size. Negative binomial GLMM.

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: totN
##       Chisq Df Pr(>Chisq)
## diet 9.7206  5    0.08355 .
## fSL  1.7134  1    0.19054
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

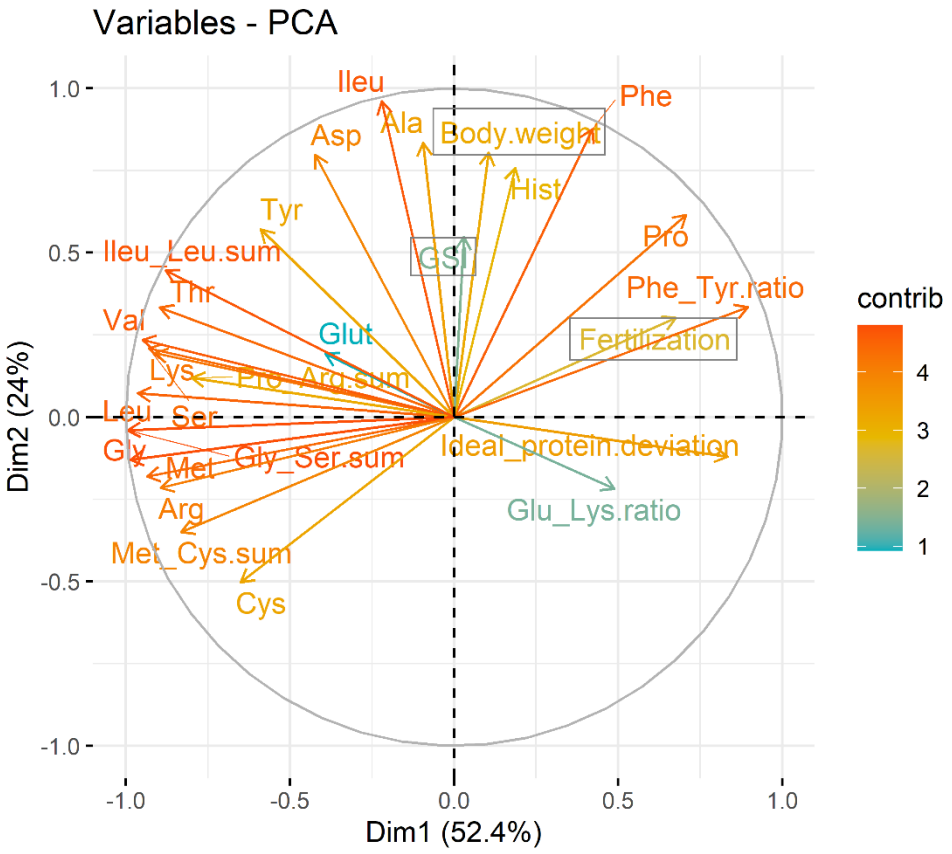
```
## $emmeans
##  diet response   SE  df asymp.LCL asymp.UCL
##  BLW      32.7 9.68 Inf     18.35      58.4
##  ALL      32.8 9.35 Inf     18.80      57.4
##  COP      16.7 4.65 Inf      9.69      28.9
##  SAK      10.1 3.09 Inf      5.56      18.4
##  SKR      23.7 6.72 Inf     13.55      41.3
##  wild     13.6 5.04 Inf      6.59      28.1
##
## Confidence level used: 0.95
## Intervals are back-transformed from the log scale
##
## $contrasts
##  contrast   ratio    SE  df z.ratio p.value
##  BLW / ALL  0.997 0.381 Inf -0.008  1.0000
##  BLW / COP  1.958 0.821 Inf  1.601  0.5979
##  BLW / SAK  3.234 1.485 Inf  2.557  0.1080
##  BLW / SKR  1.384 0.561 Inf  0.802  0.9672
##  BLW / wild 2.406 1.161 Inf  1.820  0.4528
##  ALL / COP  1.964 0.802 Inf  1.651  0.5642
##  ALL / SAK  3.244 1.440 Inf  2.651  0.0854
##  ALL / SKR  1.389 0.553 Inf  0.824  0.9632
##  ALL / wild 2.414 1.142 Inf  1.863  0.4253
##  COP / SAK  1.652 0.659 Inf  1.258  0.8078
##  COP / SKR  0.707 0.281 Inf -0.873  0.9529
##  COP / wild 1.229 0.556 Inf  0.456  0.9975
##  SAK / SKR  0.428 0.180 Inf -2.017  0.3326
##  SAK / wild 0.744 0.348 Inf -0.633  0.9886
##  SKR / wild 1.738 0.744 Inf  1.291  0.7903
##
## P value adjustment: tukey method for comparing a family of 6 estimates
## Tests are performed on the log scale
```

SUPPORTING INFORMATION S31: Statistical results of Binomial GLMM for fertilization rate.

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: binomial  ( logit )
## Formula: fr ~ diet + (1 | tankF)
##    Data: repr
##
##      AIC      BIC   logLik deviance df.resid
##    697.9    712.2   -343.0    685.9       74
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -4.9863 -1.2966 -0.0183  1.2233  8.7643
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  tankF  (Intercept) 0.06424  0.2535
## Number of obs: 80, groups:  tankF, 10
##
## Fixed effects:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept)   0.4684     0.1980   2.366 0.018001 *
## dietALL      -0.7284     0.2798  -2.603 0.009230 **
## dietCOP      -0.7470     0.2965  -2.519 0.011753 *
## dietSAK      -1.1759     0.3227  -3.644 0.000268 ***
## dietSKR      -1.0589     0.2909  -3.640 0.000273 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##         (Intr) ditALL ditCOP ditSAK
## dietALL -0.708
## dietCOP -0.668  0.473
## dietSAK -0.614  0.435  0.410
## dietSKR -0.682  0.483  0.455  0.419
```

```
## $emmeans
##  diet  prob     SE  df asymp.LCL asymp.UCL
##  BLW  0.615 0.0469 Inf     0.520     0.702
##  ALL  0.435 0.0486 Inf     0.344     0.532
##  COP  0.431 0.0541 Inf     0.329     0.538
##  SAK  0.330 0.0563 Inf     0.230     0.448
##  SKR  0.357 0.0488 Inf     0.267     0.457
##
## Confidence level used: 0.95
## Intervals are back-transformed from the logit scale
##
## $contrasts
##  contrast  odds.ratio    SE  df z.ratio p.value
##  BLW / ALL       2.07 0.580 Inf  2.603  0.0697
##  BLW / COP       2.11 0.626 Inf  2.519  0.0863
##  BLW / SAK       3.24 1.046 Inf  3.644  0.0025
##  BLW / SKR       2.88 0.839 Inf  3.640  0.0025
##  ALL / COP       1.02 0.302 Inf  0.063  1.0000
##  ALL / SAK       1.56 0.504 Inf  1.388  0.6354
##  ALL / SKR       1.39 0.404 Inf  1.138  0.7862
##  COP / SAK       1.54 0.518 Inf  1.272  0.7082
##  COP / SKR       1.37 0.419 Inf  1.017  0.8477
##  SAK / SKR       0.89 0.295 Inf -0.353  0.9967
##
## P value adjustment: tukey method for comparing a family of 5 estimates
## Tests are performed on the log odds ratio scale
```

SUPPORTING INFORMATION S32



SUPPORTING INFORMATION S32: Principal component analyses of dietary amino acids, growth and reproduction in *Nothobranchius furzeri*. Caution must be taken while interpreting negative correlations among amino acids. An increase above the necessary level does not necessarily elicit a positive physiological response. The amino acids that are rather limited then play a more important role (see, Heger & Frydrych, 2019). Some amino acids cannot be stored in the body above a certain level due to risk of toxicity (e.g. sulphur-containing amino acids; (Brosnan and Brosnan, 2006))

SUPPORTING INFORMATION S33: Statistical results of Cox mixed effects model of egg survival to 30 days post fertilization.

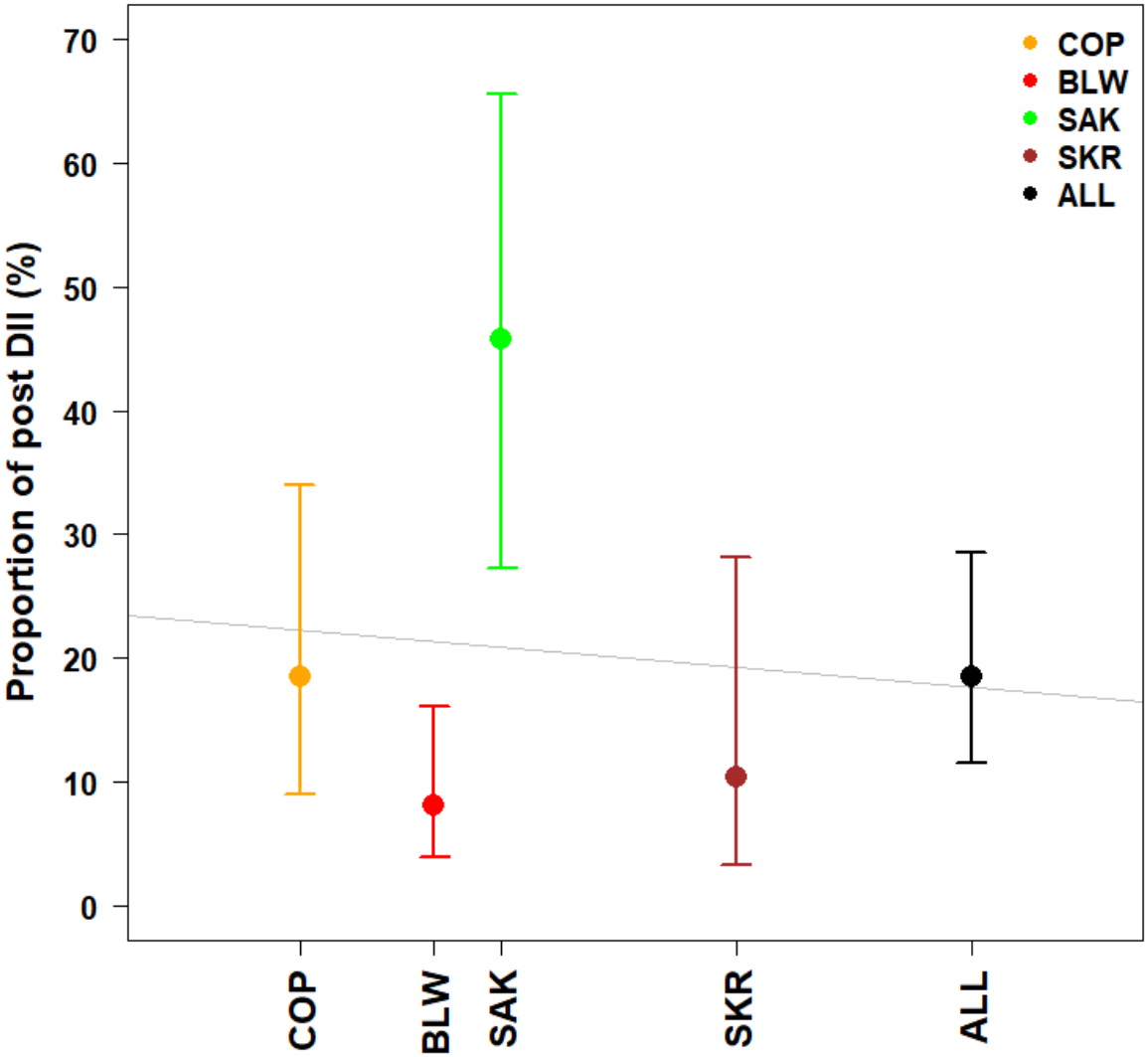
```
## Cox mixed-effects model fit by maximum likelihood
##   Data: esr
##   events, n = 329, 599
##   Iterations= 11 58
##                     NULL Integrated    Fitted
## Log-likelihood -1990.586  -1981.486 -1977.513
##
##                   Chisq   df          p   AIC    BIC
## Integrated loglik 18.20 5.00 0.00270680  8.20 -10.78
##  Penalized loglik 26.15 5.91 0.00019374 14.32  -8.12
##
## Model:  Surv(dpf, surv, type = "right") ~ diet + (1 | replicate)
## Fixed coefficients
##               coef exp(coef)  se(coef)    z      p
## dietALL 0.02625475  1.026602 0.2078880 0.13 0.9000
## dietCOP 0.25443928  1.289738 0.2242912 1.13 0.2600
## dietSAK 0.50533146  1.657535 0.2569174 1.97 0.0490
## dietSKR 0.62621547  1.870518 0.2222373 2.82 0.0048
##
## Random effects
##  Group     Variable  Std Dev    Variance
##  replicate Intercept 0.14364461 0.02063377
```

```
## $emmeans
##  diet response     SE  df asymp.LCL asymp.UCL
##  BLW     0.832 0.1013 Inf     0.655      1.06
##  ALL     0.854 0.0984 Inf     0.681      1.07
##  COP     1.073 0.1691 Inf     0.788      1.46
##  SAK     1.379 0.2842 Inf     0.921      2.07
##  SKR     1.556 0.2431 Inf     1.146      2.11
##
## Confidence level used: 0.95
## Intervals are back-transformed from the log scale
##
## $contrasts
##  contrast  ratio    SE  df z.ratio p.value
##  BLW / ALL 0.974 0.203 Inf -0.126  0.9999
##  BLW / COP 0.775 0.174 Inf -1.134  0.7883
##  BLW / SAK 0.603 0.155 Inf -1.967  0.2823
##  BLW / SKR 0.535 0.119 Inf -2.818  0.0389
##  ALL / COP 0.796 0.176 Inf -1.031  0.8410
##  ALL / SAK 0.619 0.158 Inf -1.884  0.3261
##  ALL / SKR 0.549 0.120 Inf -2.737  0.0488
##  COP / SAK 0.778 0.208 Inf -0.937  0.8826
##  COP / SKR 0.690 0.162 Inf -1.584  0.5077
##  SAK / SKR 0.886 0.236 Inf -0.454  0.9912
##
## P value adjustment: tukey method for comparing a family of 5 estimates
## Tests are performed on the log scale
```

SUPPORTING INFORMATION S34: Statistical results of Binomial Generalized Mixed Effects Model for developmental stage (prior DII or DII/post DII).

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: binomial  ( logit )
## Formula: d2_3 ~ diet + (1 | replicate)
##    Data: egdev
##
##      AIC      BIC   logLik deviance df.resid
##    242.5    264.2   -115.2    230.5      269
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -0.9231 -0.4765 -0.3395 -0.2950  3.3896
##
## Random effects:
##  Groups    Name        Variance Std.Dev.
##  replicate (Intercept) 0.002592 0.05091
## Number of obs: 275, groups:  replicate, 10
##
## Fixed effects:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept) -1.480458   0.285369  -5.188 2.13e-07 ***
## dietBLW     -0.956379   0.478422  -1.999  0.04561 *
## dietCOP     -0.007605   0.507961  -0.015  0.98805
## dietSAK      1.312644   0.491067   2.673  0.00752 **
## dietSKR     -0.680508   0.666200  -1.021  0.30703
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##         (Intr) ditBLW ditCOP ditSAK
## dietBLW -0.550
## dietCOP -0.562  0.309
## dietSAK -0.534  0.307  0.300
## dietSKR -0.361  0.217  0.203  0.211
```

```
## $emmeans
##  diet   prob     SE  df asymp.LCL asymp.UCL
##  ALL  0.1854 0.0431 Inf    0.1151     0.285
##  BLW  0.0804 0.0296 Inf    0.0384     0.161
##  COP  0.1842 0.0631 Inf    0.0902     0.340
##  SAK  0.4581 0.1032 Inf    0.2723     0.656
##  SKR  0.1033 0.0577 Inf    0.0329     0.281
##
## Confidence level used: 0.95
## Intervals are back-transformed from the logit scale
##
## $contrasts
##  contrast  odds.ratio    SE  df z.ratio p.value
##  ALL / BLW      2.602 1.245 Inf  1.999  0.2664
##  ALL / COP      1.008 0.512 Inf  0.015  1.0000
##  ALL / SAK      0.269 0.132 Inf -2.673  0.0580
##  ALL / SKR      1.975 1.316 Inf  1.021  0.8456
##  BLW / COP      0.387 0.225 Inf -1.635  0.4746
##  BLW / SAK      0.103 0.059 Inf -3.974  0.0007
##  BLW / SKR      0.759 0.555 Inf -0.377  0.9957
##  COP / SAK      0.267 0.158 Inf -2.233  0.1674
##  COP / SKR      1.960 1.473 Inf  0.895  0.8987
##  SAK / SKR      7.339 5.426 Inf  2.696  0.0546
##
## P value adjustment: tukey method for comparing a family of 5 estimates
## Tests are performed on the log odds ratio scale
```



SUPPORTING INFORMATION S35: Egg development stage after 30 days post fertilization when incubated at 25° C in Yamamoto solution. Means and error bars (95% confidence intervals) are Binomial Generalised Mixed effects Model estimates. Grey line represents relation to protein concentration. Cop – Coppens Orange, BLW – bloodworms, SAK – SAK MIX, SKR – Skretting Vitalis, ALL – Aller Infa. X axis is sorted in accordance with protein concentration in each food.

SUPPORTING INFORMATION S36A: Overview of results (comparisons) of nutritional parameters in relation to diet (thus parameters which are not related to specific diets (i.e. age dependent amount of food consumed) are not shown). Different letters indicate significant differences, if not stated otherwise. NA = data/results not available.

	FCR	Diet dependent amount of consumed food	Sex-specific amount of consumed food*
Wild	NA	A	D
Bloodworm	B	B	E
Aller	A	A	F
Skretting	A	A	E
Coppens	A	A	E
SAK	A	A	F

* D - higher consumption by females, E - higher consumption by males, F - no difference.

SUPPORTING INFORMATION S36B: Overview results for Growth, condition and other somatic parameters. Different letters indicate significant differences. NA = data/results not available. Initial body mass was estimated at 19 dph. Juvenile growth was compared as body mass at 29 dph. (bm) = body mass (g). M/F = diet dependency of parameter for males and females separately (It does not mean how male and female differ in parameters).

Diet	Initial (bm)	Juvenile growth (bm)	Age dependent body mass M/F	Age dependent body size M/F	TGC	Surv.	Bone deform.	HSI M/F	Gut length	Hepa. vacuol.	Body cond.	Visceral fat score
Wild	NA	NA	NA/NA	NA/NA	NA	NA	A	B/A	A	B	A	NA
Bloodworm	A	AB	AB/AB	AB/ABC	AB	A	A	A/A	A	A	B	A
Aller	A	AB	A/A	A/A	A	A	B	B/A	A	AB	A	AB
Skretting	A	B	A/AB	A/BC	AB	A	AB	B/AB	A	AB	AB	AB
Coppens	A	C	A/A	A/AB	AB	A	AB	B/B	A	A	AB	B
SAK	A	BC	B/B	B/C	B	A	B	B/C	A	B	AB	C

SUPPORTING INFORMATION S36C: Overview of results for reproductive parameters.

Diet	GSI	Fecundity	Fertilization rate	Egg survival	Egg development
Wild	AB	AB	NA	NA	NA
Bloodworm	AB	A	A	A	A
Aller	A	A	AB	A	AB
Skretting	AB	AB	B	B	AB
Coppens	AB	AB	AB	AB	AB
SAK	B	B	B	AB	B

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